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VALIDATION AND ESTIMATION OF THE INCERTAINTY OF THE SPADNS METHOD FOR DETERMINING FLUORIDE IN WATER

VALIDAÇÃO E ESTIMATIVA DA INCERTEZA DO MÉTODO SPADNS PARA DETERMINAÇÃO DE FLUORETO EM ÁGUAS

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Resumo

A importância de demonstrar a qualidade de medições químicas está cada vez mais exigida. Dados analíticos não confiáveis podem comprometer conclusões e tomadas de decisão gerando prejuízos, podendo muitas vezes colocar em perigo a saúde pública. Para garantir que um método analítico gere informações confiáveis ele deve passar por uma avaliação criteriosa antes de ser colocado em uso, chamada de validação, ou confirmação quando o método já for reconhecido internacionalmente. O objetivo deste trabalho foi confirmar o método de determinação de fluoreto na matriz água (Método Colorimétrico Spadns 4500 F^- D do SMWW). Os parâmetros avaliados foram: seletividade, linearidade, faixa de trabalho, limite de detecção, limite de quantificação, recuperação, precisão e incerteza. Em relação à seletividade, observou-se que a turbidez interfere quando apresenta valores iguais ou acima de 2,15 NTU e o alumínio interfere negativamente em quaisquer concentrações. Com a remoção da turbidez através de filtração à vácuo com filtro de 0,45 µm e aguardando 4 horas antes de realizar a leitura para inibir a interferência do alumínio, foi possível demonstrar a correlação entre os coeficientes angulares. Os LD e LQ da curva foram, respectivamente, de 0,04 e 0,07 mg/L de F^-. A faixa linear de trabalho determinada foi entre 0,1 e 2,0 mg/L de F^-, e a curva-padrão apresentou coeficiente de correlação maior do que 0,99, que demonstra a linearidade da metodologia. As precisões, repetibilidade e precisão intermediária, superiores a 90% foram consideradas adequadas. As recuperações foram de 97%, nas três

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faixas avaliadas. A incerteza expandida estimada foi de ±0,03 mg/L. Foi possível demonstrar e concluir que a metodologia apresenta resultados confiáveis e adequados para o propósito.

Palavras-chave: Fluoreto. Validação de métodos. Incerteza de medição.

Abstract

The importance of demonstrating the quality of chemical measurements is increasingly demanded. Unreliable analytical data can jeopardize conclusions and decision making, causing harm, and can often endanger public health. To ensure that an analytical method generates reliable information it must undergo a careful evaluation before being put to use, called validation, or confirmation when the method is already internationally recognized. The objective of this work was to confirm the method of determination of fluoride in the water matrix (Colorimetric Method Spadns 4500 F-D from SMWW). The parameters evaluated were: selectivity, linearity, working range, detection limit, quantification limit, recovery, precision and uncertainty. Regarding selectivity, it was observed that turbidity interferes when it presents values equal to or above 2.15 NTU and aluminum interferes negatively at any concentration. With the removal of turbidity through vacuum filtration with a 0.45 µm filter and waiting 4 hours before taking the reading to inhibit aluminum interference, it was possible to demonstrate the correlation between the angular coefficients. The LD and LQ of the curve were, respectively, 0.04 and 0.07 mg/L of F^-. The linear working range determined was between 0.1 and 2.0 mg/L of F^-, and the standard curve showed a correlation coefficient greater than 0.99, which demonstrates the linearity of the methodology. Accuracy, repeatability and intermediate precision, greater than 90%, were considered adequate. Recoveries were 97% in the three ranges evaluated. The estimated expanded uncertainty was ±0.03 mg/L. It was possible to demonstrate and conclude that the methodology is reliable and adequate for the purpose.

Keywords: Fluoride. Method validation. Measurement uncertainty.

1. INTRODUCTION

For more than 70 years, epidemiological studies have provided the basis for the use of fluoride in preventing dental cavities. Some countries have adopted water fluoridation programs and reductions in cavities prevalence have been recorded and attributed to wide use of fluoride. The main advantages of water fluoridation are: the prevention of dental caries throughout life, the large number of people benefited by this process and for being considered affordable compared to other means (WHELTON et al., 2019).

Although there is a consensus on the relationship between the use of fluoride and the reduction of dental caries, it can be said that there is an ideal dose, because when ingested in high concentrations it becomes a toxic substance, being dental fluorosis the main effect. Other chronic and more serious effects are joint and muscle pain, resulting from skeletal fluorosis (POLLICK, 2018).

In Brazil, the Ministry of Health implemented the first water fluoridation system in 1953, being mandatory from 1974 with the Federal Law Nº 6.050. It was regulated that the adequate concentration of fluorine in water in the Brazilian territory is in accordance with the average maximum annual temperatures of each region (RAMIRES; BUZALAF, 2007). In the state of Santa Catarina, Ordinance nº. 421/16, establishes that the concentration range of fluoride ion in water intended for human consumption is from 0.7 mg/L to 1.0 mg/L (SANTA CATARINA, 2016).

It is noteworthy that water fluoridation is a process that requires control, in view of the existence of an ideal dose recommended by law, which public supply companies must follow, in order to provide water to the population with the quality requirements met.

Thus, unreliable analytical data can jeopardize conclusions and decision making, generating losses, and can often endanger public health. According to the Brazilian norm ABNT NBR ISO IEC 17025:2017, a laboratory must validate methods that have been modified in some way and demonstrate the influence of these changes, in addition to demonstrating the performance characteristics that can be: measurement range, accuracy, measurement uncertainty of results, detection limit, quantification limit, selectivity of method, linearity, repeatability, reproducibility, robustness, among others. Methodology validation, as defined by the International Vocabulary of Metrology – IVM, is a "verification in which the specified requirements are adequate for an intended use" (INMETRO, 2012). In addition to carrying out the validation process, laboratories must identify contributions to measurement uncertainty, including all the significant contributions (ABNT NBR ISO IEC 17025, 2017).

The present work is a methodology validation for determining fluoride in waters that are treated and distributed to the cities served in the state of Santa Catarina, in addition to estimating the measurement uncertainty of the method.

The determination of fluoride by the Spadns Colorimetric Method is based on the reaction between fluoride and a spadns dye complex (red) with zirconium. Fluoride reacts with the complex by dissociating a portion of it into a colorless complex anion (ZrF_6^{2-}) and the dye.

As the amount of fluoride increases, the color produced becomes progressively lighter. The reaction rate between fluoride and zirconium ions is strongly influenced by the acidity of the reaction mixture. If the proportion of acid in the reactant is increased, the reaction can be done almost instantly. According to this methodology, there are several interferents that, if present in excess, the sample must be distilled (SMWW, 2017).

Having a validated methodology for fluoride determination that presents reliable results is essential to ensure the supply of drinking water for the population with a fluoride concentration in the recommended range, in view of the benefits and harms mentioned above.

Thus, the aim of this study was to validate the method for determining fluoride in water (Spadns Colorimetric Method) evaluating the following parameters: selectivity, linearity, working range, detection limit, quantification limit, recovery, precision, and uncertainty.

2. MATERIAL E METHODS

100 mg/L of fluoride certified reference material (CRM) supplied by Inorganic Ventures were used as standard (Lot K2-F652018). The reagent solution called spadns was prepared using hydrochloric acid (Vetec, Lot 03474), sodium arsenite (Neon, Lot 36216) and zirconium oxychloride (Neon, Lot 35722), and the Spadns (Qhemis, Lot 13092272). The preparation procedure was carried out as follows:

Solution A: 958 mg of Spadns (2-(parasulfophenylazo)-1,8-dihydroxy-3,6-naphthalene disulfonate sodium) were weighed, then dissolved in type 1 water in a 500 mL volumetric flask. **Solution B:** 133 mg of zirconium oxychloride octahydrate and 500 mg of sodium arsenite were dissolved in about 25 mL of type 1 water. 350 mL of hydrochloric acid were added. It was filled up to 500 mL with type 1 water. At the end the two solutions (A and B) were mixed.

The type 1 water used to prepare the solutions in this study was obtained from Direct-Q equipment (Merck). Analyses were performed using the Pocket Colorimeter II (Hach), using 10 mL of sample (or standard) and 2 mL of Spadns reagent solution.

The results were statistically evaluated using the Microsoft Office 2016 Excel software. The merit parameters (validation) were evaluated following the guideline document (DOC-CGCRE-008) and are described below:

2.1 Selectivity

The influence of interfering agents was evaluated: turbidity and aluminum. For this, a fluoride standard of 1.00 mg/L was prepared and contaminated for different turbidity scales (1.0; 2.0; 3.0; 5.0 and 10.0 NTU). To assess the influence of aluminum, standard 1.0 mg/L of fluoride was prepared and contaminated with different concentrations of aluminum (0.1; 0.2, 0.5 and 1.0 mg/L) and readings were taken in different times (1, 2, 3, 4 and 5 hours). All tests were performed on seven replicates.

After studying the effects of interferents and adapting the methodology to remove turbidity and inhibit aluminum interference, two curves were prepared to compare the slopes (angular coefficients). One of the curves (curve A) was prepared with type 1 water and MRC fluoride standard. The concentrations of the studied solutions were 0.0; 0.2; 0.4; 0.8; 1.0; 1.4; 1.6; 1.8 and 2.0 mg/L. The other curve (curve B) was prepared with the same fluoride standards mentioned above, but they were contaminated with turbidity (between 15 and 22 NTU) and aluminum concentration 0.2 mg/L, thus simulating the worst-case sample that the laboratory can receive. These standards were then vacuum filtered with 0.45 µm filter paper. Each standard was analyzed in 7 replicates, and the reading was performed after 4 hours of reaction, to remove aluminum interference.

2.2 Linearity

Fluoride solutions were prepared at concentrations of 0.0; 0.03; 0.2; 0.4; 0.8; 1.0; 1.4; 1.6; 1.8 e 2.0 mg/L, from 1000 mg/L MRC fluoride standard. Then, the reactions were prepared, in triplicate, and the readings were performed on the Pocket Colorimeter II.

2.2.1 Homoscedasticity

The Grubbs test was performed to verify the existence of outliers. Subsequently, the homoscedasticity of the methodology was calculated by applying the Cochran test using the formula below:

Where:
S^2max is the biggest variance
$\sum S^2$ is the sum of all variances

After confirming the homoscedasticity of the method, a simple linear regression equation was used.

2.2.2 Working Range

Following the guideline document (DOC-CGCRE-008) the approximate linear range and the upper and lower limits of the working range were initially identified. Curves were prepared from the analysis of Real Concentration x Measured Concentration (mean of 3 measurements).

2.3 Detection limit

The detection limit (DL) was assessed using sample blank readings. For this, eight blanks were prepared with type 1 water. Using one of the blanks, the equipment, Pocket Colorimeter II, was reset, and then the readings of the other blanks were taken. DL was evaluated using the following equation:

$$\mathrm{LD} = \overline{X} + t_{(n-1,\,1-\alpha)} \,.\, s$$

Where:
$\overline{X}$: mean of blank sample values;
t: abscissa of the Student distribution, depending on sample size and confidence level;
s: standard sample deviation of blanks sample.

2.4 Quantification Limit

The quantification limit (QL) was also evaluated through the blank sample tests, using the blank results, previously tested to calculate the QL. The QL was determined using the following equation:

$$\mathrm{LQ} = \overline{x} + 5.s$$

Where:
$\overline{X}$: mean of blank sample values;
s: standard sample deviation of blanks sample.

After determining the QL value, a solution with MRC standard at the calculates concentration was prepared to test and assess the precision, through 7 replicates.

2.5 Recovery

Recovery was assessed using a real sample (tap water) containing 0.53 mg/L of fluoride. Then, a fluoride standard at three levels of concentrations (0.4, 0.8 and 1.2 mg/L of F-) was added to this sample and 7 replicates were performed for each concentration. The equation used was:

$$\text{Recovery (\%)} = \left(\frac{C1-C2}{C3}\right).\ 100$$

Where:
C1: Concentration of the analyte in the fortified sample;
C2: Concentration of the analyte in the unfortified sample;
C3: Concentration of the analyte added to the sample.

Precision was evaluated in two ways: repeatability and intermediate precision.

2.6.1 Repeatability

Repeatability was evaluated under the following conditions: same analyst, same equipment and in a short period of time. Seven replicates were performed for each analyst (three), and repeatability was evaluated using the variation coefficient (VC), also known as Relative Standard Deviation (RSD), which was calculated using the equation:

$$\text{VC} = \left(\frac{SD}{MCD}\right).\ 100$$

Where:
SD: Standard deviation;
MCD: Mean concentration determined.

2.6.2 Intermediate Precision

Intermediate precision was evaluated under the following conditions: same measurement procedure, same equipment, different time periods and different analysts (three). The equation used was:

$$Spi_{(j,k)} = \sqrt{\frac{1}{t(n-1)}\sum_{j=1}^{t}\sum_{k=1}^{n}\left(y_{jk}-\overline{y_j}\right)^2}$$

Where:
t: total of tested samples;
n: total tests performed per sample;
j: sample n°, j = 1, t
k: sample test n ° j, k = 1, n
y_{jk}: result value k for sample j
$\bar{y}_j$: arithmetic mean of the results of sample j.

2.7 Measurement Uncertainty

For uncertainty estimation, the components were identified using the Ishikawa diagram. For that, the components related to the calibration certificates of the equipment and glassware, the MRC fluoride standard certificate and the components relates to the validation parameters were considered: precision, accuracy and the calibration curve. To perform the calculations, the

ISO/GUM Guide (JCGM, 2008) and the guidance document DOQ-CGCRE-019 where used (CGCRE, 2019). The sources of uncertainty were determined with a 95% confidence level.

3. RESULTS E DISCUSSION

3.1 Selectivity

Selectivity demonstrates the degree to which the method quantifies the analyte in the presence of other analytes or interferers, wich may enhance or inhibit the quantification of the analyte (CGCRE, 2020).

3.1.1 Influence of turbidity

The results of the readings of the fluoride standard used for the study can be seen in Table 1 and the turbidity in Table 2. The results of fluoride measurements after preparation of samples with turbidity can be evaluated in Table 3.

Table 1. Fluoride 1.00 mg/L standard readings to perform the studies

	Fluoride 1.00 mg/L standard readings							
	Reading 1	Reading 2	Reading 3	Reading 4	Reading 5	Reading 6	Reading 7	Mean
	1.04	1.05	1.05	1.04	1.05	1.05	1.04	1.05

Table 2. Turbidity readings of samples prepared with 1.05 mg/L fluoride standard and turbidity at different concentrations to perform the studies

Turbidity Standarts	Turbidity readings in NTU							
	Reading 1	Reading 2	Reading 3	Reading 4	Reading 5	Reading 6	Reading 7	Mean
1 NTU	1.10	1.09	1.09	1.20	1.10	1.10	1.00	1.10
2 NTU	2.10	2.19	2.19	2.20	2.18	2.10	2.11	2.15
3 NTU	2.99	3.01	3.00	3.01	3.03	3.04	3.03	3.02
5 NTU	4.70	4.69	4.68	4.69	4.68	4.68	4.69	4.69
10 NTU	9.95	9.89	9.96	9.92	9.96	9.94	9.95	9.94

Observing the data in Table 3, it was found that turbidity influences the fluoride results, causing a negative effect on the final result. This reduction may be linked to the adsorption of fluoride ion on the surface of the organic matter present in the sample. The real difference of means was verified through statistical analysis, t-Student test considering a confidence level of 95%. It was identified interferences determining fluoride by the Spadns methodology in water samples with turbidity equal to or above 2.15 NTU. In this way, it is recommended to remove prior to analysis.

Table 3. Results of samples prepared with 1.05 mg/L fluoride standard and turbidity at different concentrations

Turbidity Standard	Fluoride readings in mg/L							
	Reading 1	Reading 2	Reading 3	Reading 4	Reading 5	Reading 6	Reading 7	Mean
1.10 NTU	1.05	1.04	1.03	1.04	1.04	1.04	1.03	1.04
2.15 NTU	1.06	1.09	1.07	1.09	1.08	1.08	1.08	1.08
3.02 NTU	1.01	1.01	1.01	1.00	1.02	1.02	1.01	1.01
4.69 NTU	0.98	0.99	0.97	0.99	0.98	0.99	1.01	0.99
9.94 NTU	0.93	0.93	0.91	0.94	0.94	0.94	0.94	0.93

3.1.2 Influence of aluminum

In the fluoride in water determination by the Spadns Colorimetric Method, aluminum above 0.1 mg/L produces a negative error and must be compensated through the reaction time before reading (SMWW, 2017).

Table 4 shows the results of the influence of aluminum in the determination of fluoride (1.05 mg/L) over time. The results clearly show that, over time, it is possible to recover the fluoride value. Furthermore, it was observed that the fluoride response is in versely proportional to the aluminum concentration present in the sample in relation to time. This fact is due to the competition between aluminum and fluoride ions in the center of the formed complex.

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Table 4. Influence of aluminum at different concentrations

	Time	Reading 1	Reading 2	Reading 3	Reading 4	Reading 5	Reading 6	Reading 7	Mean
Standard 0.1mg/L Al + 1mg/L F^-	Immediate	0.99	1.00	0.99	1.00	0.99	0.99	0.99	0.99
	After 1h	0.97	0.99	0.99	1.01	1.01	1.00	0.99	0.99
	After 2h	1.02	1.04	1.03	1.05	1.03	1.03	1.03	1.03
	After 3h	1.01	1.02	1.04	1.02	1.04	1.04	1.02	1.03
	After 4h	1.00	1.01	1.01	1.01	1.00	1.01	0.99	1.00
	After 5h	1.00	1.01	1.01	1.03	1.02	1.01	0.99	1.01
Standard 0.2mg/L Al + 1mg/L F^-	Immediate	0.90	0.91	0.93	0.94	0.94	0.94	0.92	0.93
	After 1h	0.95	0.96	0.98	0.98	0.98	0.97	0.97	0.97
	After 2h	0.99	1.00	1.02	1.02	1.03	1.02	1.02	1.01
	After 3h	1.02	1.02	1.02	1.02	1.02	0.99	0.98	1.01
	After 4h	0.98	0.98	1.02	1.01	1.01	1.01	1.01	1.00
	After 5h	0.98	1.00	0.99	1.02	1.03	1.02	1.02	1.01
Standard 0.5mg/L Al + 1mg/L F^-	Immediate	0.74	0.71	0.75	0.74	0.72	0.73	0.77	0.74
	After 1h	0.89	0.89	0.91	0.89	0.88	0.88	0.92	0.89
	After 2h	0.96	0.95	0.97	0.96	0.95	0.96	0.98	0.96
	After 3h	0.98	0.96	0.98	0.97	0.97	0.96	1.00	0.97
	After 4h	0.97	0.97	1.00	0.99	0.98	0.98	1.01	0.99
	After 5h	0.96	0.98	1.00	1.00	0.99	0.99	1.03	0.99
Standard 1.0mg/L Al + 1mg/L F^-	Immediate	0.38	0.41	0.4	0.42	0.42	0.39	0.42	0.41
	After 1h	0.76	0.80	0.8	0.79	0.81	0.77	0.80	0.79
	After 2h	0.88	0.91	0.91	0.92	0.92	0.90	0.91	0.91
	After 3h	0.93	0.95	0.95	0.96	0.96	0.94	0.95	0.95
	After 4h	0.94	0.97	0.98	0.98	0.99	0.96	0.97	0.97
	After 5h	0.95	0.99	0.98	0.98	1.02	0.98	0.98	0.98

Despite observing that fluoride concentration recovers over time, it is not possible to affirm statistically, with 95% confidence, that 100% of the original fluoride concentration (1.05 mg/L) is recovered.

Using the equipment available in the laboratory, the samples were prepared by vacuum filtration using a 0.45 µm filter to remove turbidity. The results shown in Table 4 indicate that for safety, a waiting time of 4 hours should be followed in order to eliminate aluminum interference. Then, the selectivity was evaluated by comparing the two analytical curves, one constructed without interfering agents (Table 5) and the other prepared with the addition of turbidity and aluminum, previously treated as proposed (Table 6).

Table 5. Fluoride readings of standards prepared only with reagente water and MRC fluoride standard (without the interferentes)

Fluoride standards	Curve without interferers– Curve A			
	Reading 1	Reading 2	Reading 3	Mean
0.20	0.20	0.23	0.24	0.21
0.40	0.40	0.42	0.43	0.42
0.80	0.80	0.80	0.80	0.80
1.00	1.00	0.99	1.00	1.00
1.40	1.40	1.38	1.42	1.43
1.60	1.60	1.63	1.65	1.63
2.00	1.80	1.83	1.77	1.80

Table 6. Fluoride readings of standards prepared with interferentes .(0.2 mg/L of aluminum and turbidity between 15 and 22 NTU) after 4 hours of reaction before taking the reading

Fluoride standards	Curve with interferences – Curve B							
	Reading 1	Reading 2	Reading 3	Reading 4	Reading 5	Reading 6	Reading 7	Mean
0.20	0.15	0.14	0.14	0.18	0.17	0.16	0.15	0.16
0.40	0.37	0.39	0.40	0.39	0.39	0.38	0.38	0.39
0.80	0.82	0.83	0.82	0.83	0.83	0.82	0.81	0.83
1.00	1.01	1.00	1.00	1.02	1.01	1.02	0.98	1.01
1.40	1.38	1.35	1.40	1.40	1.38	1.40	1.38	1.38
1.60	1.6	1.59	1.61	1.59	1.61	1.60	1.59	1.60
2.00	1.97	1.99	1.95	1.97	1.98	1.95	1.98	1.97

Curve A, prepared without interference, presented an R2 equal to 0.9996 (Figure 1) while curve B, prepared with interferences, presented an R2 equal to 0.9988 (Figure 2).

Figure 3 shows the two analytical curves plotted simultaneously. It was found that the slopes of both linear regression curves did not differ significantly, indicating that there is no matrix effect and proving the efficiency of the proposed sample filtration methodology when it presents turbidity above 2 NTU and waiting 4 hours before performing the reading (for removal of interfering aluminum). T-Student Test for angular coefficients (p = 0.37) proves what was detected visually: the straight lines are parallel.

Figure 1. Curve A (without interferers)

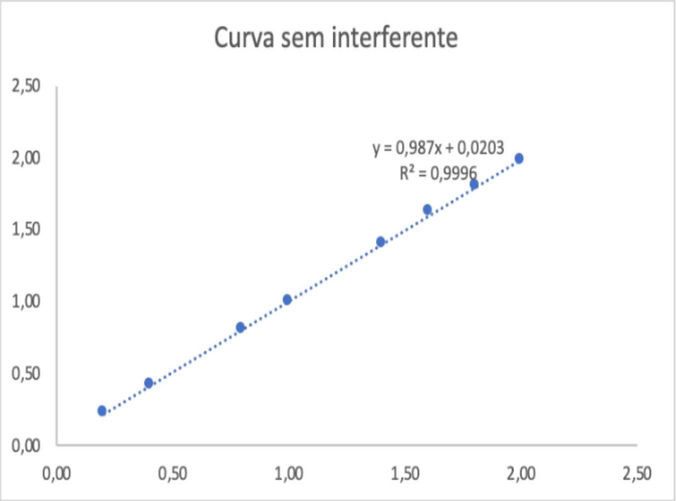


Figure 2. Curve B (with interferers – aluminum and turbidity)

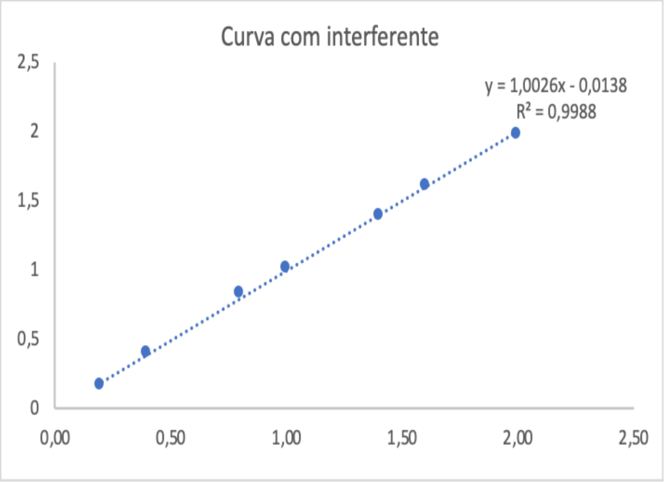
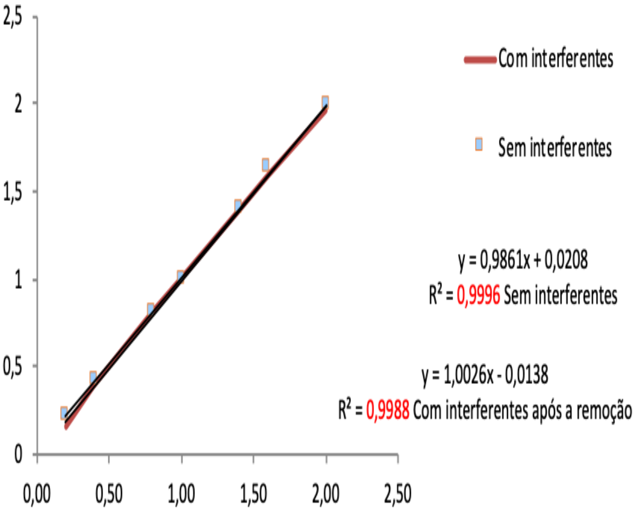


Figure 3. Parallelism of curves A and B demonstrated above



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3.2 Linearity and Working Range

The linearity of a quantitative analytical method is its ability to obtain results directly proportional to the concentration of the analyte in the sample, within a range, while the working range is the range between the lowest and highest concentration of analyte of the method, in which precision, accuracy and linearity demonstrate an acceptable level (CGCRE, 2020).

Depending on the level of demand regarding the issue of results, values greater than or equal to 0.99 are recommended. In this work was chosen for considering correlation coefficient (r) equal to or greater than 0.99 as adequate to assess the linearity of the method. It is noteworthy that the individual analysis of this coefficient is not conclusive in relation to linearity, so the analysis of residuals is also recommended.

Following DOQ-CGCRE-008 guidelines, the absence of outliers was verified before performing the linear regression, through the Grubbs test. The results are shown in Table 7.

The absence of outliers is confirmed through the individual analysis of the calculated values with the tabulated critical value (Gcritical 2.11). Comparing the results shown in Table 8, it was identified that the calculated values are smaller than the Gcritical, confirming the absence of outliers.

Table 7. Grubss calculation

Level	[C] mg/L	Readings			Mean	Grubss		
		Reading 1	Reading 2	Reading 3		G1	G2	G3
1	0.03	0.040	0.030	0.040	0.040	0.5774	-1.155	0.577
2	0.20	0.230	0.240	0.210	0.230	0.2182	0.8729	-1.091
3	0.40	0.420	0.430	0.420	0.420	-0.577	1.1547	-0.577
4	0.80	0.800	0.800	0.800	0.800	-0.816	-0.816	-0.816
5	1	0.990	1.000	1.000	1.000	-1.155	0.5774	0.577
6	1.4	1.380	1.420	1.430	1.420	-1.134	0.378	0.756
7	1.6	1.630	1.650	1.630	1.630	-0.577	1.155	-0.577
8	1.8	1.830	1.770	1.800	1.800	1.000	-1.000	0.000
9	2.00	1.960	1.960	1.980	1.960	-0.577	-0.577	1.155

Table 8. Blank readings (DL calculation)

Blank	Readings (mg/L)
1º Blank	0.02
2º Blank	0.02
3º Blank	0.02
4º Blank	0.02
5º Blank	0.03
6º Blank	0.03
7º Blank	0.00
Mean	0.02
Standard deviation	0.01
n	7
gl	6
t	3.14
t (table)	1.943180281
Angular coefficient	0.9889
DL = 0.02 + 1.943180281 * 0.01	
DL = 0.039431803	

If outliers are present, it is recommended to delete the value and perform a new test to replace the value that was out. In addition to the Grubbs test, the homoscedasticity of the data that generated the analytical curve was verified using the Cochram test. For this, the variances of the individual results were calculated and, through the equation mentioned in section 2.2.1, the value for the Ccalculated was defined. The same is compared with the tabulated value, being identified that: Ccalculated < Ctabulated, in this case we have homogeneity of variances, indicating that simple linear regression using unweighted ordinary minimums can be used.

After the above-mentioned analyses, a calibration curve was created from the data obtained in the readings in mg/L, obtaining in the end a value for the linear correlation coefficient of R2=0.9918, shown in Figure 4.

The residuals of the calibration curves were also evaluated (Figures 5 and 6). They showed randomness between the points, not being described through predictable trends. At the end, it was identified that the working range was between 0.07 and 2.0 mg/L.

Figure 4. Linearity of the curve for fluoride determination

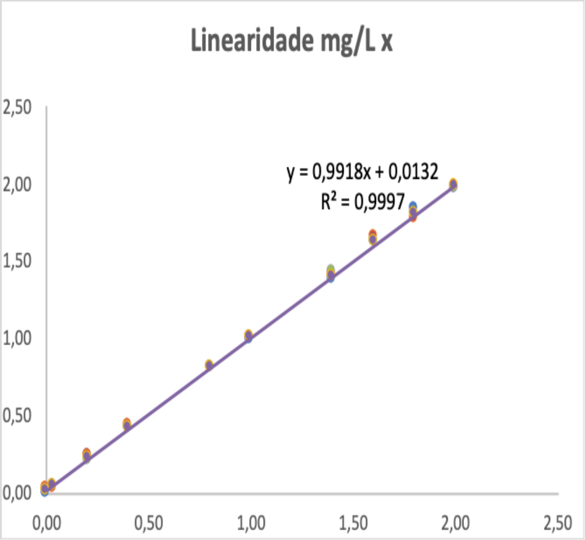


Figure 5. Residues readings in mg/L

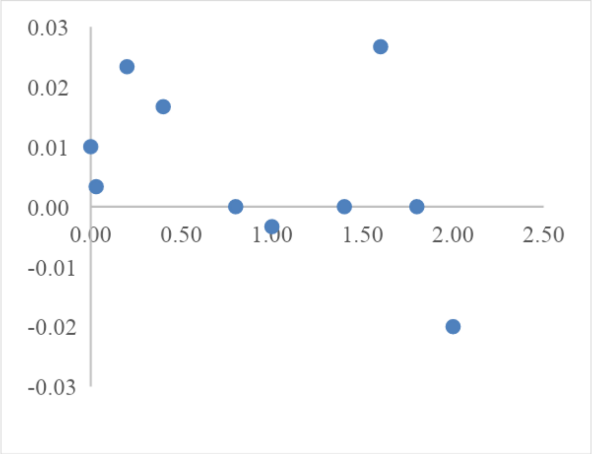
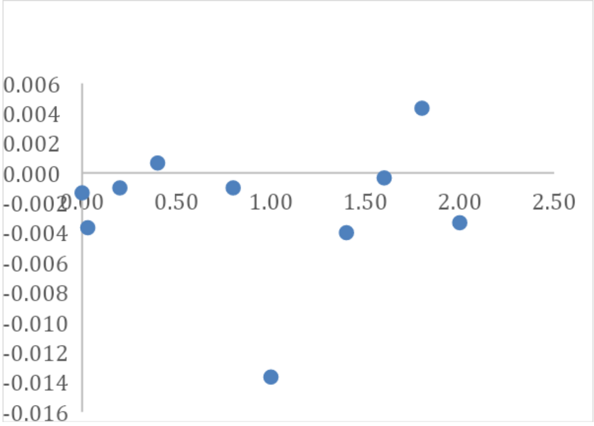


Figure 6. Residues absorbance readings



3.3 Detection Limit and Quantification Limit

The detection limit represents the smallest amount of analyte in the sample that can be detected but not quantified. While the limit of quantification is the smallest amount of analyte in the sample that can be quantitatively determined with defined and acceptable reliability such as precision and accuracy (CGCRE, 2020).

The results obtained from the preparation of the seven blanks are shown in Table 8.

The DL was calculated using the equation cited in Section 2.3, resulting in 0.004 mg/L. The QL was calculated using the equation cited in Section 2.4 resulting in 0.07 mg/L. This result demonstrates that the method is sensitive to quantify the levels of fluoride concentration present in water samples in accordance with Ordinance nº 421/2016 of the State of Santa Catarina. After determining the quantification limit, tests were performed to prove it, shown in Table 9.

Table 9. QL proof

Standard 0.10 mg/L	Standard 0.07 mg/L
0.09	0.06
0.09	0.07
0.10	0.08
0.11	0.08
0.11	0.07
0.11	0.08
0.09	0.09
Mean 0.10	Mean 0.076
Standard deviation 0.01	Standard deviation 0.005345
VC(%) 10	VC(%) 7.6

The tests confirmed that the value of both 0.10 and 0.07 mg/L had a recovery value between 80% and 110%, having a coefficient of variation less than 11%, indicating that both values can be used as QL, however, the value of 0.07 mg/L represents greater safety for the laboratory.

3.4 Recovery

Recovery is used to assess the accuracy of the method. It can be estimated by analyzing samples spiked with known amounts of the same analyte, also called spike (CGCRE, 2020).

The recovery value obtained was greater than 97% in the three ranges evaluated, as can be seen in Table 10.

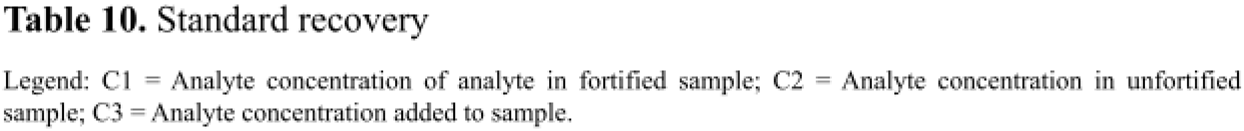


Table 10. Standard recovery

Legend: C1 = Analyte concentration of analyte in fortified sample; C2 = Analyte concentration in unfortified sample; C3 = Analyte concentration added to sample.

This result is considered acceptable given that, according to criteria established by the AOAC, a recovery between 80 and 110% is recommended (CGCRE, 2020).

3.5 Precision

Accuracy is defined by the degree of agreement between measured values obtained by repeated measurements on the same object. It can be evaluated in three ways or conditions: repeatability, intermediate precision and reproducibility. The repeatability condition involves using the same measurement procedure, same operator, same location, same conditions, repeated measurements on the same object over a short period of time. The intermediate precision condition comprises the same measurement procedure, the same location and repeated measurements on the same object over an extended period of time where other variables are included, such as different analysts or equipment (INMETRO, 2012).

3.5.1 Repeatability

The repeatability for each analyst was evaluated using the individual coefficient of variation. It appears that the CV value in all cases is less than 1%, and the acceptance criterion established by the AOAC is 11%. The results of each analyst can be seen in Table 11. It was verified that, regardless of the analyst, the levels of precision are in accordance with those established by the laboratory, indicating that everyone is able to perform the test in question.3.5.2 Intermediate precision

The standard deviation of the intermediate precision, in which the analysts and time were varied, was calculated according to the equation described in Section 2.6, with a value of 0.008. The results can be seen in Table 12.

Subsequently, ANOVA test was applied to assess the equivalence between the analysts. After the test, the probability p value identified was 0.73, which means that there is no difference between the analysts' means, considering a significance level of 95%.

3.6 Measurement uncertainty

According to IVM measurement, uncertainty is a “non-negative parameter that characterizes the dispersion of values assigned to a measurand, based on the information used” (INMETRO, 2012).

The individual components considered in the uncertainty calculation are shown in Table 13. The precision-related uncertainty component evaluated 7 replicates of readings of the MRC 1.00 mg/L fluoride standard. The component of the uncertainty related to accuracy was determined using the recovery value, obtained from the fortification of a real sample with a standard solution of MRC fluoride 0.80 mg/L.

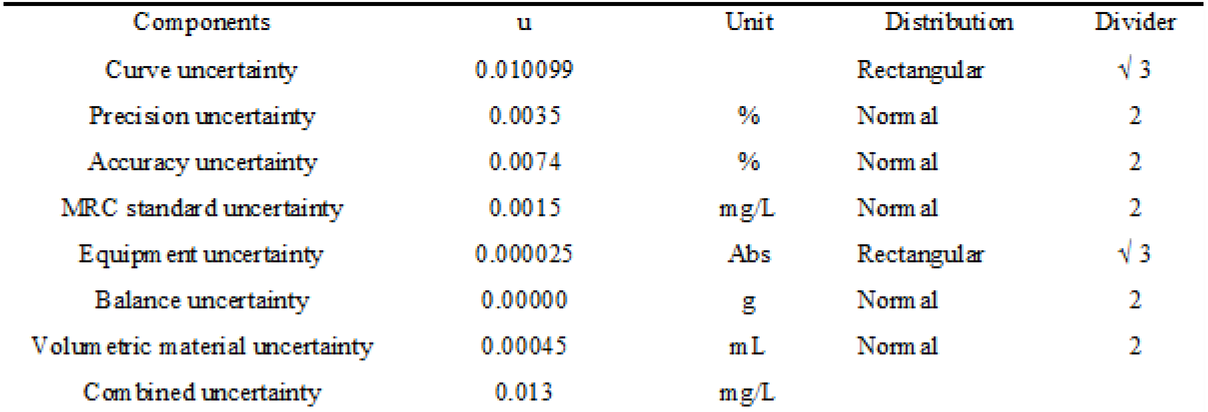
Table 11. Repeatability represented by 7 replicates of the fluoride standard for each analyst

Analyst	R1	R2	R3	R4	R5	R6	R7	Mean	Intermediate Precision
1	1.05	1.06	1.04	1.05	1.04	1.04	1.03	1.04	0.008
2	1.04	1.03	1.05	1.05	1.05	1.04	1.05	1.04	
3	1.05	1.04	1.05	1.05	1.04	1.05	1.05	1.05	

Table 12. Fluoride standard readings to determine intermediate precision

Analyst	R1	R2	R3	R4	R5	R6	R7	Mean	Intermediate Precision
1	1.05	1.06	1.04	1.05	1.04	1.04	1.03	1.04	0.008
2	1.04	1.03	1.05	1.05	1.05	1.04	1.05	1.04	
3	1.05	1.04	1.05	1.05	1.04	1.05	1.05	1.05	

Table 13. Components used to estimate measurement uncertainty

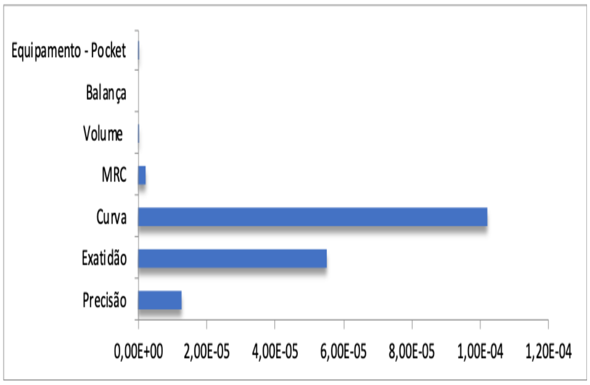


Components	u	Unit	Distribution	Divider
Curve uncertainty	0.010099		Rectangular	√ 3
Precision uncertainty	0.0035	%	Normal	2
Accuracy uncertainty	0.0074	%	Normal	2
MRC standard uncertainty	0.0015	mg/L	Normal	2
Equipment uncertainty	0.000025	Abs	Rectangular	√ 3
Balance uncertainty	0.00000	g	Normal	2
Volumetric material uncertainty	0.00045	mL	Normal	2
Combined uncertainty	0.013	mg/L		

The calculated value for the expanded uncertainty, considering a confidence level of 95%, a coverage factor k of 2.00 and all individual components of uncertainty was ± 0.03 mg/L for the value of 1.00 mg/L of F^-.

Figure 7 shows the influence of each individual component of uncertainty. It appears that the most relevant component in the method is related to the calibration curve and the accuracy of the method, which is mainly related to the training of the analyst and the purity of the reagents. This analysis makes it possible to diagnose in which situations more attention and consideration should be given in the execution of the method, in order to obtain results with safe uncertainty values and reliable results.

Figure 07. Major contributors to spadns method measurement uncertainty



4. CONCLUSIONS

The method presented in the study exhibited validation parameters suitable for determining the fluoride concentration in water for human consumption, evidenced through the performance in relation to selectivity parameters, linearity/working range, detection limit, quantification limit, recovery and precision (repeatability and intermediate precision).

The study highlighted the importance of carrying out the validation process so that the methodology is really understood, how to understand how the interferers act and how the internal variations of the methodology occur, varying the analysts who perform the tests.

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