Validating a High Performance Liquid Chromatography-Ion Chromatography (HPLC-IC) Method with Conductivity Detection After Chemical Suppression for Water Fluoride Estimation

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Abstract Abstract A variety of methods, including the Ion Selective Electrode (ISE), have been used for estimation of fluoride levels in drinking water. But as these methods suffer many drawbacks, the newer method of IC has replaced many of these methods. The study aimed at (1) validating IC for estimation of fluoride levels in drinking water and (2) to assess drinking water fluoride levels of villages in and around Vellore district using IC. Forty nine paired drinking water samples were measured using ISE and IC method (Metrohm). Water samples from 165 randomly selected villages in and around Vellore district were collected for fluoride estimation over 1 year. Standardization of IC method showed good within run precision, linearity and coefficient of variance with correlation coefficient $R^2 = 0.998$. The limit of detection was 0.027 ppm and limit of quantification was 0.083 ppm. Among 165 villages, 46.1% of the villages recorded water fluoride levels >1.00 ppm from which 19.4% had levels ranging from 1 to 1.5 ppm, 10.9% had recorded levels 1.5–2 ppm and about 12.7% had levels of 2.0–3.0 ppm. Three percent of villages had more than 3.0 ppm fluoride in the water tested. Most (44.42%) of these villages belonged to Jolarpet taluk with moderate to high (0.86–3.56 ppm) water fluoride levels. Ion Chromatography method has been validated and is therefore a reliable method in assessment of fluoride levels in the drinking water. While the residents of Jolarpet taluk (Vellore district) are found to be at a high risk of developing dental and skeletal fluorosis.

Keywords Fluoride · Fluoride estimation · Drinking water · Ion chromatography

Introduction

Fluorosis is a major public health problem, and it is endemic in about 24 countries worldwide which have recorded high levels of Fluoride in drinking water [1]. Fluoride in nature exists in the ionic forms of fluorspar and rock phosphate. As it is abundant and universal in the earth's crust, all water sources contain varying concentrations of fluoride. Therefore, the major source of fluoride for humans is drinking water [2]. The World Health Organization (WHO) and the Bureau of Indian standards have set the permissible maximum limit of fluoride in drinking water to be 1.5 ppm (mg/L) and 1.0 ppm respectively [3]. But some states in India have markedly high levels of fluoride. Water fluoride levels of more than 1.5 ppm have been recorded in about 17 states in India [4]. About 62 million people in India, including 6 million children; have developed Fluorosis, due to the consumption of drinking water with high levels of fluoride. The states which are worst affected are Rajasthan, Bihar, Jharkhand, Punjab, Chhattisgarh, Maharashtra, Haryana, Andhra Pradesh, Karnataka and Tamil Nadu [5].

Water analysis is a vital part of the chemical analysis of environmental samples. The development of the newer methods of water analysis and improving the existing methods is the major task at hand for an analytical chemist. A variety of methods have been used for the chemical analysis of fluoride levels in drinking water including colorimetry, titrimetry, Ion selective electrode (ISE) and amperometric titrations [6]. However many of the methods are not specific and suffer from interference, limited

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sensitivity and are often difficult to automate. The new technology of HPLC-IC which has replaced many wet chemical methods in water analysis was used in the present study to assess the levels of fluoride in drinking water [7].

Materials and Methods

165 villages in Vellore district (Tamil Nadu) were randomly selected for this cross-sectional study. Water samples were collected from these villages, which included Ranipet, Arcot, Ambur, Vaniyambadi, Gudiyatham, Alan-gayam, Jolarpet, Natrampalli, Katpadi and Kaniyambadi over a period of 1 year (2015–2016) and fluoride levels were assessed by using HPLC-IC [8]. (Metrohm, AG, Switzerland)

Ion Chromatography Methodology

Principle: This method separates molecules through ion exchange depending on their respective charges.

Method: The water sample is inserted into a stream of carbonate-bicarbonate eluent, and it then passes through a number of ion exchangers. The fluoride ions are separated depending on their relative affinities for the strongly basic anion exchanger (guard and separator columns). After separation of fluoride ions, they are then passed through the hollow fabrication exchanger membrane (fiber suppressor) or micro membrane suppressor which has a strong acidic solution (regenerant solution) passing through it continuously. In the micro membrane suppressor, the fluoride ions are converted to the highly conductive acid while the carbonate-bicarbonate eluent is converted to weakly conductive carbonic acid. The separated anions are only measured in the acid form using conductivity. Retention time is compared to standards for identification and quantification is via measurement of peak height or peak area [9].

Equipment Details

881 Compact IC pro Metrohm and 889 IC sample center (autosampler) was used.

Settings Used in the HPLC-IC System

Analytical and Guard columns both contained used poly-vinyl alcohol with quaternary ammonium groups. The Eluent was 3.2 mmol/L of Sodium Carbonate with 1 mmol/L of Sodium Hydrogen Carbonate. The Suppressor solution was 50 mmol/L of Sulphuric acid. Run time was 27 min per injection. Flow rate was 0.7 ml/min, sample

volume injected was 20 mcL (μ l). Maximum pressure (P max) was 15 MPa. The column was held at room temperature. The Detector used was a Conductivity detector and Fluoride retention time was 3.2 min.

The stock fluoride (anion standard) solution of 10 ppm (10 mg/L) was supplied by Fluka Sigma, Bangalore. This was diluted with pure deionised water to give working standard solutions of 10.0, 5.0, 1.0, 0.5 and 0.1 ppm of fluoride which are stable for 24 h after preparation.

Any large particles (>0.45 microns) in the water samples as well as the large particles (>0.20 microns) in the reagent solutions must be removed by filtration filtered prior to analysis as they may cause damage to the instruments columns and the flow systems.

Ion Selective Electrode (ISE) Methodology

Drinking water fluoride was assayed using ISE (Thermo Scientific Orion 4 star pH/ISE bench top pH meter). To perform the analysis, an equal volume of sample (1.0 ml) and TISAB buffer were placed in a non-glass beaker, and mixed. The fluoride electrode was cleaned by rinsing with deionized water, dried and placed into the sample beaker with continuous stirring, until a stable reading was obtained. The concentration of the sample was displayed on the meter. Calibration solutions from 1.0 to 10.0 ppm of fluoride were used.

Method Comparison

49 paired samples of drinking water with a fluoride concentration range of 0.5 to 10.2 ppm were measured using both (ISE) and the HPLC-IC (Figs. 1, 2, 3, 4, 5).

Fig. 1 Calibration graph of measurement fluoride by HPLC-IC. The Correlation coefficient (R^2) for this calibration graph was 0.999, and the relative standard deviation was 2.4% with a Slope of 0.266 and an intercept of -0.016

Fig. 2 Method comparison: HPLC-IC with Ion Selective Electrode. The Correlation coefficient (R^2) is 0.998, the slope 1.02 and the intercept -0.076 using 49 values

Fig. 3 The comparison of IC and ISE methodology using Bland Altman plot. There is no discernible bias within the Bland Altman plot and all differences fall within $\leq 4\%$ of the mean values shown

Results

HPLC-IC Evaluation

LOD may be expressed as:

$$\text{LOD} = \frac{3.3\sigma}{S}$$

where σ the standard deviation of the response, S the slope of the calibration curve.

The estimate of σ is based on standard deviation of blank run $10\times$ consecutively [10].

Limit of detection (LOD) was 0.027 ppm (equivalent to mg/L).

The quantification limit (QL) may be expressed as:

$$\text{QL} = \frac{10\sigma}{S}$$

Fig. 4 Fluoride levels in the drinking water of 165 villages in Vellore district using HPLC-IC

where σ the standard deviation of the response, S the slope of the calibration curve. The estimate of σ based on standard deviation of blank run $10\times$ consecutively [10].

Limit of quantification (LOQ)—0.083 ppm.

The calibration range was determined to be from 0.1 to 100 ppm.

The Correlation coefficient (R^2) for x calibration graphs was 0.999, and the relative standard deviation was 2.4% with a Slope of 0.266 and an intercept of -0.016 .

Precision Within run precision was determined using 10 repeat analyses values with fluoride standards of 0.5, 1.0, 2.0, 5 and 10 ppm. The following results were obtained: (Table 1).

Linearity Samples were assayed under various dilutions using the deionised water in which the fluoride levels were undetectable using Ion Chromatography by HPLC-IC (Table 2).

Discussion

Drinking water is primarily used for human consumption but is also used for other household purposes. Drinking water is considered to be safe when it poses no danger to human health. As mentioned earlier, a variety of methods used for analysis of water samples can be replaced by HPLC-IC [6]. Validation of the method showed a good within run precision, linearity and %CV. The Limit of Detection (LOD) in our analysis was found to be 0.027 ppm but another study had found it to be lower

Fig. 5 Map of the Vellore district Tamil Nadu highlighting the taluks of Natrampalli and Jolarpet

Table 1 Within-run precision

Sample no.	Mean	SD	% CV
1	0.53	0.019	3.60
2	1.06	0.064	5.98
3	2.03	0.077	3.80
4	5	0.1	2.10
5	10.5	0.2	2.00

(0.01 ppm) [11]. However the Metrohm application note states it to be 0.05 ppm [12]. This shows that our method LOD agrees well with other users. The limit of quantification (LOQ), in the IC method, was found by us to be 0.083 ppm, while other studies have stated it to be higher (0.125 ppm) [11]. The present study is the first to compare drinking water fluoride levels using HPLC-IC with the existing ISE method. The HPLC-IC method compared well.

Table 2 Linearity table

Sample no.	Dilution	Observed (O) F value (ppm)	Expected (E) F value (ppm)	(O × 100)/E
1	8 in 8	2.6	N/A	N/A
	4 in 8	1.38	1.3	106
	2 in 8	0.78	0.65	DIFF—0.13 ppm
	1 in 8	0.49	0.33	DIFF—0.16 ppm
2	8 in 8	5	N/A	N/A
	4 in 8	2.57	2.5	100
	2 in 8	1.36	1.25	109
	1 in 8	0.77	0.63	DIFF—0.14 ppm
3	8 in 8	11.6	N/A	N/A
	4 in 8	5.56	5.58	95.8
	2 in 8	2.6	2.9	90
	1 in 8	1.4	1.45	96
4	8 in 8	32.3	N/A	N/A
	4 in 8	14.7	16.15	91
	2 in 8	7.55	8.05	94
	1 in 8	3.67	4.02	93

Observed and expected values within $\pm 10\%$ or ± 0.2 ppm are acceptable. The linearity was acceptable in all the dilutions of samples

N/A not applicable, DIFF Difference

Though HPLC-IC is a novel method of assessing water fluoride levels, but it has drawbacks. For serum analysis, the fluoride has to be extracted prior to analysis and also chloride has to be removed from the sample. For urine analysis as well, chloride and other cations need to be removed from the urine sample prior to analysis as especially chloride was found to interfere with the analysis [13]. The IC method requires sample extraction before analysis which makes it more costly, and time consuming. There is a long run time of 27 min for each sample. To overcome this, an automated sample injection system was used. There was no change in the fluoride concentration following dilution but the interference of Chloride cannot be removed by this process. These drawbacks make HPLC-IC more tedious to perform than the ISE method which is much more straightforward.

It was found that among 165 villages, 46.06% of the villages recorded water fluoride levels above the recommended Indian standards (<1.00 ppm). Earlier studies have also noted higher ground water fluoride levels in 4 Panchayats of the Alangayam and Thirupattur blocks of Vellore districts. The ground water fluoride levels ranged from 0.43 to 4.59 ppm [14]. In contrast, another study done at the Walaja block in Palar river basin had recorded water fluoride levels within the permitted WHO and Indian ranges (from 0.6 to 0.8 ppm) [15]. A high fluoride intake in these areas poses a threat of fluorosis to the population.

Conclusion

In conclusion, HPLC-IC is a reliable method for assessment of fluoride levels in the drinking water, but it has a few limitations. It is known to be expensive and tedious as it requires chloride and other cations to be extracted from serum and urine samples prior to analysis. The run time is 27 min per sample, which means the throughput of samples is low. For this reason the analysis was performed overnight using an automated sample injector. This makes the ISE method much easier to perform. Using the HPLC-IC method for analysis, Jolarpet and Natrampalli taluks of Vellore district was found to have high levels of ground water fluoride which may predispose the population to develop dental and skeletal fluorosis.

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