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Comparison of Gas Chromatographic and Ion Selective Electrode Methods for Measuring Fluoride in Urine

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Fluorine in the human body may exist in both inorganic and organic (covalently bound) forms. The inorganic fraction is clearly the most relevant for assessing human exposure to and utilization of environmental fluoride. As a means to determine fluoride exposure, testing urine samples is both a noninvasive procedure and easier to perform than other tests involving blood, hair, nails, bone or saliva (Ophaug, 1994). There are three basic requirements for determining fluoride concentrations in any types of samples and using any method: (1) fluoride must be separated from the interfering substances that are present or that the interferences be suitably suppressed by appropriate procedures, (2) final fluoride concentration must be adequate for reliable measurement by the method employed, and (3) the experimental fluoride blank must be significantly smaller than the amount of fluoride involved in the analysis. ISE (ion selective electrode) method is commonly used for estimating free fluoride ion in biological samples. Tsul (1972) used the ISE method to measure fluoride concentrations in human urine and found that it is convenient and suitable for testing large quantities of samples. Chiba, et al.(1980) used aluminum monofluoride (AIF) molecular absorption spectrometry with a fluoride ion selective electrode to measure fluorine in urine and blood serum. They found that AIF molecular absorption and ISE methods were consistent with each other when testing urine. However, using AIF molecular absorption to determine resulted in values 2-10 times higher than using the ISE method. Chiba et al. suggested that this discrepancy be due to the existence of some protein-bound fluorine in blood. An additional method, the hanging-drop fluoride electrode method (HDFE method) as described by Venkateswarlu (1974), can also be used and therefore not readily available. Venkateswarlu indicated the fluoride from a large sample is extracted as a fluorosilane (and free from interfering substance) into an organic layer and then back-extracted as fluoride ions into a few micro-liter of an aqueous phase. The detection limits using HDFE method was 0.01-0.02 ppm and the recovery efficiencies was ranged from 95.5-100.5% among biological samples. Ikenishi, et al.(1988) used the GC method to determine fluoride concentrations in biological samples. Ikenishi selected toluene as a vehicle for trimethylchlorosilane (TMCS) instead of carbon tetrachloride which was used by Fresen, et al.(1968) because the latter is toxic and produces interfering peaks and requires a long time (over 60 minutes) to measure fluoride. The retention time of TMFS in the GC chromatogram was considerably short.

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Recoveries of fluoride for monkey plasma, rat plasma, and human control serum were 91.9% and 95.3%, and 88.1%, respectively. Recovery efficiencies for urine were not mentioned in this study. However, Ikenishi's results were consistently lower than those found by other similar studies. Ikenishi's method used to measure fluoride levels in humans (0.53 µg/mL average) which was lower than the average level of Japanese (0.72± 0.46 µg/mL). However, Ikenishi's study failed to account for the low results which were due to the inability to separate TMFS (trimethylfluorosilane) peak from other interferences. And because Ikenishi also failed to provide detailed information regarding quality control procedures, the current study was undertaken to evaluate the accuracy and reproducibility of using GC method in comparison with ISE method to measure fluoride concentrations in urine.

MATERIALS AND METHODS

Urine specimens were collected in 250 mL plastic bottles containing 0.2 g of EDTA. They were then stored at 4 °C and analyzed within three days. A 10 mg/L commercial fluoride standard was purchased from Orion (Cat. No. 040908). The standard reference material for freeze-dried urine (SRM2671) certified for fluoride supplied from the U.S. National Bureau of Standards. Both sets of standards were diluted using TISAB(Total Ionic Strength Adjustment Buffer) (Orion 940911).

Fluoride concentrations were determined using the GC method as described by Ikenishi et al. (1988). This method is suggested for the assay of more than 5 ng/mL of fluoride in biological samples. In his study, Ikenishi states that TMCS combines with free fluoride to form TMFS which can be measured by GC-FID. A Hewlett-Packard 5890 Series II gas chromatography/FID with a J&W fused silica capillary column (DB-1, 30m/0.25mm/0.25µm) was used for this purpose. The injector temperature was 160°C and the detector temperature was 200°C. The column temperature was held at 35°C for the first three minutes after injecting and then increased by 25°C/min to 200°C, which was maintained for an additional 20 minutes. The assay was conducted by first transferring 1 mL of the sample into a 15 mL centrifuge tube along with 4 mL of H_2O, 1 mL of 3 N $HClO_4$, and 0.5 mL of two percent (v/v) a TMCS-toluene solution. The tube was stoppered and shaken for 10 min. After centrifugation at 3000 rpm(Kubota 5100, Japan) for 10 min, 5 µL from the toluene layer was injected into the GC-FID. In Figure 1, retention times for TMFS were 1.054 min using the standard solution and human urine (figure 1(a) and figure 1(c))and 1.055 min using the freeze-dried urine standards (figure l(b)). Results from the GC method were not affected by other substances in the urine. Due to the short retention times in the gas chromatogram and presence of interference, the resolution was not very clear. As a result, it was difficult to accurately calculate the peak areas of TMFS.

Commercial standard fluoride solutions of 0.14, 0.28, 0.565, 1.125 and 2.25 mg/L were prepared and a calibration curve was created. Freeze-dried urine standards containing fluoride were also prepared and a second calibration curve of 0.11, 0.34, 0.68, 1.25 and 2.28 mg/L was also created. In Figure 2(a), the lines on the calibration curve were parallel using ISE method but on Figure 2(b) the lines were converging using GC method. This convergence is due to interference from substances in the urine, or "matrix effect," and as a result SRM standards must be

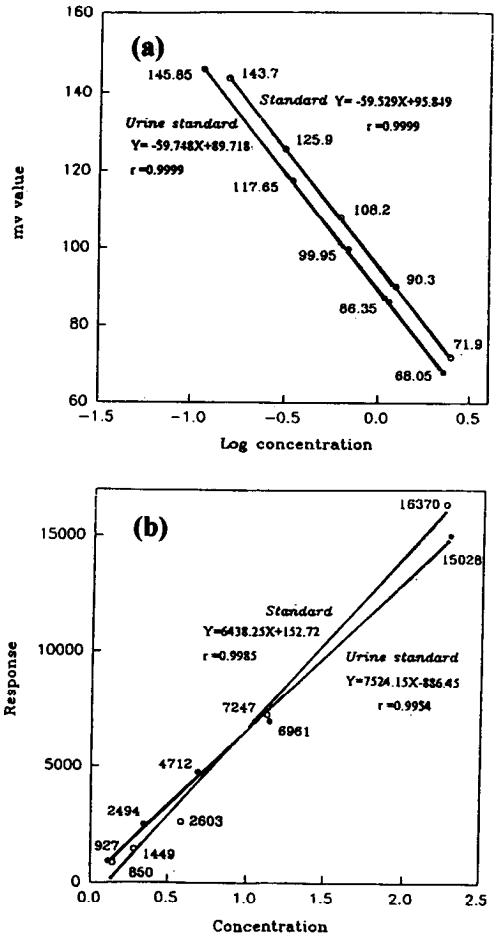

Figure 1. (a) Calibration curve of commercial and freeze-dried fluoride standards using ISE methods. (b) Calibration curve of commercial and freeze-dried fluoride standards using GC methods.

used when measuring for fluoride concentrations in human urine samples. 0.2ml of five different standard fluoride concentrations (ranging from 0.14 - 2.25 mg/L) were spiked to pool urine samples to determine reproducibility. Each concentration was tested three times. The standard reference material for freeze-dried urine was prepared in concentrations of 0.11 to 2.28 (mg/L). A 0.2ml standard reference material was used to calculate the recovery efficiency: Each sample was tested three times according to the Ikenishi procedure to determine fluoride recovery.

The ISE method has been refined to assay down to 0.01 mg/L with a high degree of accuracy. This method was performed using an Orion fluoride electrode (model 9409 BN; Orion Research, Cambridge, Mass, USA) and reference electrode (Model 9001). The reference electrode was tilled with a 3M KCl solution saturated with AgCl. Polyethylene containers were used for sampling and storage. Because measurements of fluoride ion activity in a solution is influenced by pH values, ionic strength, temperature, and extraneous ions, these factors must be strictly

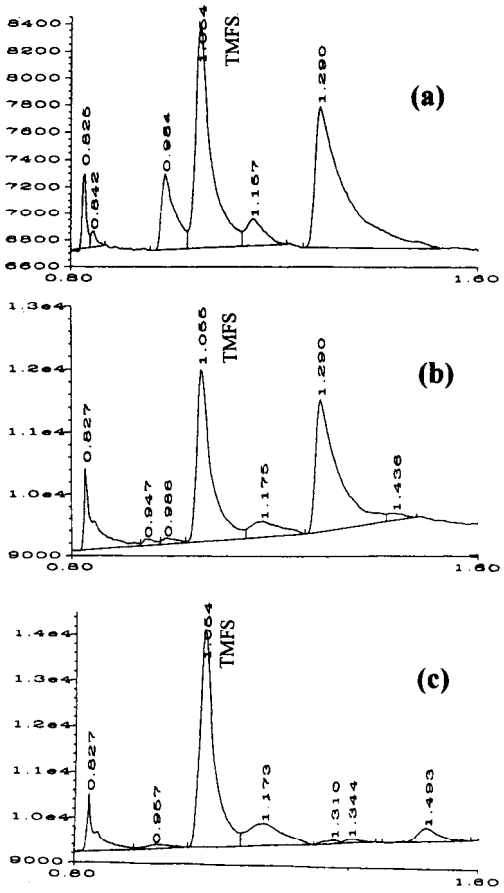

Figure 2. (a) Retention time of TMFS peak using commercial fluoride standard in gas chromatogram. (b) Retention time of TMFS peak using freeze-dried fluoride standard in gas chromatogram. (c) Retention time of TMFS peak using human mine sample in gas chromatogram.

controlled in both standard and experimental solutions to ensure precise results. When determining the reproducibility of fluoride concentrations in commercial standard, Freeze-dried urine standard and human urine, tests were performed 7 times on each sample. The coefficient of variation (CV) was calculated to be < 1%. The ISE and GC methods were tested for consistency using the three SRM concentrations (low, medium, and high) and 35 urine specimens.

RESULTS AND DISCUSSION

The human body contains both organic and inorganic fluoride compounds. Since urine is acidic, most of the fluoride in urine is inorganic. Using the GC method, CV for the retention time was less than 4.0% (Table 1). Commercial and freeze-dried fluoride standards exhibited small differences in retention times. Using chromatograms to calculate CV values for standard concentrations varied from 3% to 19%. Variations were due to the short retention times and the inability to separate other peaks from interfering. Interference resulted in difficulty to accurately determining peak areas of TMFS. In 1988, when Ikenishi experienced a

similar phenomenon, he measured the peak height of the TMFS to calculate the fluoride concentration from the calibration curve.

Table 1. Reproducibility in determination of urinary fluoride concentrations by GC method (N=3)

Amount (μg)	CV of retention time (min)	CV of concentration
0.028	0.7[a]	6.1
0.056	4.0	19.2
0.113	0.9	3.0
0.225	1.0	5.0
0.450	4.0	11.0

[a] Coefficient of Variation (%)

Results from recovery of fluoride using the GC method are described in Table 2. The recoveries ranged from 30.7% to 56.0%. However, Ikenishi, et al. (1988) performed the same procedure as described above, but stopped after the first extraction and used the GC method to measure TMFS. Recovery of plasma and serum was highly efficient using the GC method (CV was < 5%). Venkateswarlu (1974) developed the reverse-extraction technique for the separation concentration of fluoride. In an acidic environment, TMCS is added to a sample containing fluoride and the TMCS combines with the fluoride to form TMFS. A base is then added, which combines with TMFS and releases fluoride into the aqueous phase. The fluoride can then be measured free of any interfering substances using the hanging-drop fluoride electrode method. Errors in measuring of low levels of fluoride, particularly in the vicinity of the limits of measurement of the electrode (0.01 to 0.02 mg/L), are due to variations in the background ionic strength, composition among samples and standard, and the need for prolonged equipment times required (Venkateswarlu, 1983). Another reason for the inaccurate measurement was caused when TMCS reacts with fluoride in an acidic state, TMCS produces fluoro-compounds other than TMFS, such as trifluorotoluene, pentafluoropropanol and heptafluorobutanol, etc. which are readily vaporized (Venkateswarlu , 1974).

The results from using the GC and ISE methods to test three SRM freeze-dried standard samples containing urine fluoride with low, medium, and high concentrations are shown in Table 3. ISE results were consistent with concentrations from the freeze-dried standard samples. The average ratio of GC values to ISE values

Table 2. Recoveries of GC method at different standard reference material for freeze-dried urine fluoride concentrations

Amount (μg)	Recovery (%)
0.23	49.1
0.68	42.4
1.38	30.7
2.28	56.0
4.56	40.7

for the three samples was approximately 30%. Strict quality control procedures ensured the accuracy of ISE's results. The correlation coefficient for the calibration curve exceeded 0.995. The relative predicted deviation (RPD) was less than 5%. Fluoride solutions of 0.03, 0.12 and 3.26 mg/L were each tested five times. The

coefficients of variance (CV) were 5.9%, 3.1% and 1.2%, respectively. The fluoride in the suitably buffered sample solution, which responds to the fluoride ion

Table 3. Comparison of ISE and GC methods for determining urinary fluoride concentrations

SRM (mg/L)	ISE Method Mean ± SD	GC Method Mean ±: SD	Ratio (GC/ISE)
0.25	0.24 ± 0.03	0.08 ± 0.12	0.35
0.80	0.77 ± 0.02	0.23 ± 0.09	0.31
2..40	2.46 ± 0.02	0.74 ± 0.39	0.30

(N=3)

electrodes in the absence of the fluoride "unmasking" reagent such as TISAB should be considered free of ion fluoride. Any additional fluoride revealed by use of fluoride "unmasking" reagent would represent metal-complexed inorganic fluoride. There would be a chemical equilibrium between the free-fluoride ion and the complex fluoride. The ISE is sensitive only to ionic fluoride and not to the organic compound. Urine samples from 35 voluntary selected subjects were tested using both the GC and ISE methods. (Figure 3) Results showed GC concentrations were consistently lower than ISE concentrations. The correlation coefficient was 0.66. Chiba et al. (1982) used a GC atmospheric pressure helium microwave-induced plasma (MIP) emission spectrometric system to measure fluoride concentrations in urine. Fluorine can be detected in helium MIP because it is excited by metastable helium's high amounts of energy. The high level of energy easily breaks the covalent bonds. However, because GC/FID cannot produce high amounts of energy to adequately excite fluoride, this method may produce low concentrations of fluoride in urine. Venkateswarlu (1975) developed the oxygen bomb technique for determination of fluoride in serum. But this method still has two main problems that rendered the blank correction inaccurate: first, fluoride loss during open ashing of biological samples and, second, possible contamination by various amounts of extraneous fluoride. Venkateswarlu made successful recoveries of added inorganic fluoride, but such recoveries do not necessarily guarantee the accuracy of the ashing procedure, since some loss of fluoride may occur due to vaporization. Another reason is that nonionin, covalently-bound fluoride (organic F) should also be part of the total fluoride determined in the samples, but Kabadese et al. (1971) were able to recover only 21.7 to 71.3% of fluorine from selected organic fluorine compounds following open ashing. The GC method may be used to determine organic fluoride in biological samples but not accurately determine inorganic fluoride in urine. Moreover, Venkateswarlu, et al. (1990) used absorption spectrometry to determine total fluoride in biological samples. This simple method requires only one injection of the samples and can be suitably diluted with the matrix solution. If the procedure can be automated, it would save considerable amounts of time and labor.

To conclude, the GC method underestimates the true level of fluoride in urine because the TMCS does not extract all the available fluoride. The GC method is up to three times less effective than the ISE method for measuring fluoride in urine. As

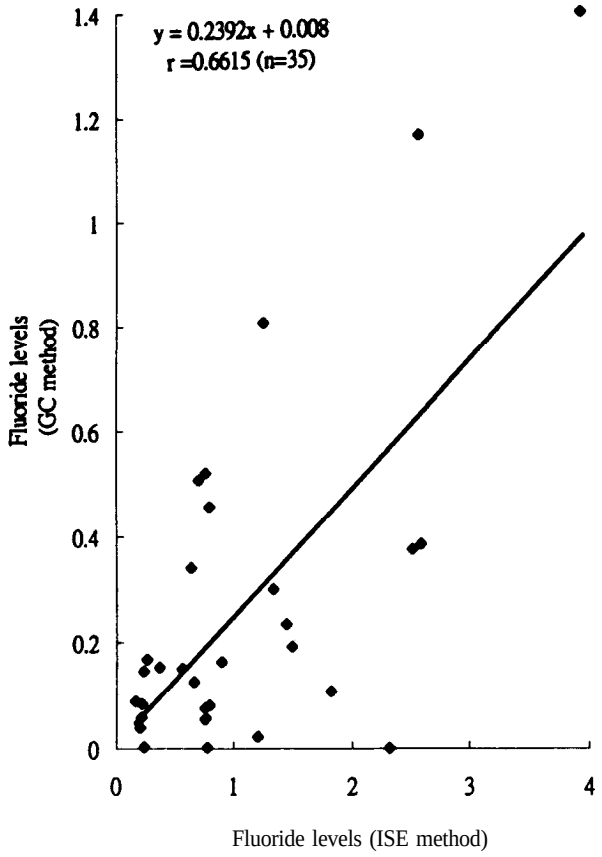

Figure 3. GC method and ion selective electrode (ISE) to determine urinary fluoride concentration (mg/L)

well as being more reliable, quicker and easier, the ISE method is well suited to studies which have large numbers of samples.. When measuring fluoride concentrations in biological specimens, the GC method may produce artificially low results which require further verification.

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