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Determination of Fluoride Ion Concentration in Reach ACT Anticavity Fluoride Rinse Using Ion Exchange Chromatography

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Chemistry 134
Principles of Quantitative Analysis

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Abstract

Fluoride ion concentration in commercial mouthrinse was determined by ion exchange chromatography. Mean fluoride ion content and standard deviation was measured at 271.3 ± 9.0 ppm. This measurement was significantly different from the concentration of fluoride reported by the mouthrinse manufacturer, 226 ppm (p < 0.05), and had a relative error of 20%. The mean mass of fluoride transferred to the body by the use of mouthrinse was calculated at 0.6 mg ± 0.1 mg. Inaccuracy of fluoride concentration measurements was attributed to chromatogram peak overlap and sample overloading. The results of this experiment suggested that ion exchange chromatography is a fast, easy, and precise method of detecting anions only when detecting concentrations within the range of a linear relationship between peak area and analyte concentration.

Introduction

The intake of fluoride ion has such substantial dental health benefits, including cavity and tooth decay prevention, that it is added to drinking water, beverages, and even food products\(^1\). Many further increase their exposure to fluoride by using other fluorinated products, such as dietary supplements and fluorinated mouthrinse. However, the safety of fluoride supplementation has been disputed, since excess fluoride ingestion can adversely affect health, causing dental fluorosis and bone weakening\(^2\). In light of these risks, the government mandates that products display their fluoride concentrations. These concerns about fluoride intake demonstrate the need for a method to accurately and precisely evaluate the fluoride concentration of products such as fluorinated mouthrinse. Such an analysis
could help to ensure that fluorinated mouthrinses are providing consumers with fluoride in adequate and safe quantities.

An ideal analytical technique for this problem is that of ion exchange chromatography. Before the advent of ion chromatography, chromatographic separations were only moderately efficient, and it was difficult to detect analytes that did not absorb UV radiation. Ion exchange chromatography, a type of high performance liquid chromatography, provides a specific analytical technique for separating anions, cations, and low molecular weight water-soluble organic acids and bases. This technique, which makes use of ion-exchange resins as stationary phases for liquid chromatography, provides chemists with an efficient and accurate method of detecting charged analytes. Though a mature technique, ion exchange chromatography continues to grow in applicability with an emphasis on analysis of new and more complex samples.

Ion exchange chromatography differs from conventional chromatography in that two columns are used. The first column consists of an ion-exchange resin bonded to inert polymeric particles. This resin has a charge that opposes the analyte’s charge. The stronger the charge on the analyte, the stronger it will be attracted to the resin, and the longer it will take to elute. Frequently, a positively charged quaternary ammonium covalently attached to the packing material is used as the stationary phase. The mobile phase is generally an ionic buffer in which pH and ionic strength are used to control elution times.

A conductivity detector would not be effective immediately after the first column, because the conductivity brought about by the electrolytic eluent overpowers the conductivity brought about by the analyte and thus lowers the sensitivity of detection. The solution to this problem is the use of a second column known as an eluent suppressor
column. This column is packed with a second ion exchange resin that converts the eluting solvent to a molecular species of limited ionization but does not affect the analyte. In an anion separation, for example, the suppressor column packing is the acidic form of the cation exchange resin. Then, sodium bicarbonate can be used as the eluting agent, resulting in the following reaction in the column:

$$\text{Na}^+_{(aq)} + \text{HCO}_3^- + \text{resin}^+ \text{H}^+ \rightarrow \text{resin}^+\text{Na}^+_{(a)} + \text{H}_2\text{CO}_3_{(aq)}$$

Because the primary ion that remains in solution is analyte, very small changes in analyte concentration can be measured by a conductivity detector. The analyte can then be quantified, assuming a linear relationship between peak area and ion concentration.

One way of determining the efficiency of a column is through analysis of band broadening. After injection of a sample, a narrow chromatographic band is broadened during its movement through the column. As band broadening increases, fewer components can be separated in a given amount of time. The peak width of individual peaks is therefore a measure of the efficiency of the column. Peak width depends on many parameters, including column length, flow rate, and particle size. In the absence of unusual interactions, a chromatographic peak should appear as a Gaussian curve with a standard deviation, $\sigma$. The efficiency of a column is then described in terms of plate number, $N$, defined as:

$$N = \left(\frac{t_R}{\sigma}\right)^2$$

where $t_R$ is the retention time of the peak being measured.

The Van Deemter equation describes band broadening as the result of three distinct sources: the multiple possible paths for an analyte through the column packing, molecular diffusion, and the effect of mass transfer between phases. Because the stationary phase resin is particulate, there are many different pathways that analyte molecules can take through the
labyrinth of resin. Peak broadening occurs because of this variance in the distance, and thus time, that each analyte molecule travels. Also, because species migrate from regions of higher concentration to lower concentration, analyte diffusion occurs laterally in the column, resulting in further peak broadening. Lastly, peaks are broadened by mass transfer between the mobile and stationary phases: the thickness of the film on the resin particles that the analyte must travel through to be adsorbed is variable, so there is variance in the amount of time required for analyte adsorption. These factors are all related to mobile phase flow rate, so it is often altered to effect optimum efficiency.

Given the effectiveness with which ion exchange chromatography can be used to detect ions in solution, this analytical tool was applied to the present experiment. The purpose of this experiment was to evaluate the accuracy and precision of ion exchange chromatography as a method for measuring fluoride ion concentration in commercial mouthrinse. In addition, this experiment aimed to determine how much fluoride is received by a user of fluorinated mouthrinse after a single rinse.

By analyzing standards of known fluoride ion concentration with ion exchange chromatography, one can create a calibration curve relating peak area to fluoride concentration. Then, mouthrinse samples can be analyzed and fitted to this curve to determine their fluoride ion composition. Determining the amount of fluoride actually retained by the body, however, is more complicated. This can be done by analyzing both mouthrinse and the contents of the discharge after the use of the product. The amount of fluoride present in a mouthrinse sample minus the amount of fluoride discharged is then a measurement of the amount of fluoride received by the consumer. Because the ion exchange chromatography method is well suited to measuring fluoride concentration, this experiment
should provide relevant information concerning the efficacy and safety of mouthrinse as a means of fluoride treatment.

**Procedure**

ACS reagent-grade NaF, concentrations 10mM and 100mM, were obtained from JT Baker. The fluorinrated mouthrinse used was Reach ACT Anticavity Fluoride Rinse, manufactured by Johnson and Johnson, with a reported fluoride ion content of 0.0226%, or 226 ppm. HPLC analyses were performed using a Dionex IonPac 4mm anion-exchange column, a GP50 gradient pump, and an ED40 electrochemical detector. All analyses used 2.7 mM Na₂CO₃/0.3 mM NaHCO₃ solvent pumped at a linear flow rate of 1.5 mL/min. The software program Dionex Peaknet 5.1 was used to integrate chromatogram peak areas.

To determine the limit of fluoride detection by ion-exchange HPLC, defined as a signal to noise ratio of 3 to 1, the spectra of low-concentration fluoride samples—19.6, 9.8, and 4.9 ppb—were taken. No sample preparation was needed for analysis of ACT mouthrinse. Three samples of unused mouthrinse were measured. Then, to determine the amount of fluoride introduced into the body through the use of mouthrinse, fluoride concentrations in known volumes of used mouthrinse were measured. Three 7.000 ± 0.001 mL aliquots of unused mouthrinse were prepared. With each aliquot, a subject rinsed his mouth for one minute and then expectorated completely. Between rinses, the subject waited 15 minutes and washed his mouth by rinsing three times with ~ 10 mL H₂O. After each aliquot was discharged, the used mouthrinse samples were allowed to stand for 20 minutes before analysis, so that any bubbles generated by the swishing process would settle.
Expectorated samples were then diluted with water to 25.00 ± 0.03 mL, and their HPLC chromatograms were taken.

To relate chromatogram data to fluoride concentration, a calibration curve of peak area versus fluoride concentration was plotted. Six standard solutions of fluoride in water, with concentrations ranging from 9 to 256 ppm, were prepared and analyzed by HPLC. The area of each chromatogram's fluoride peak was plotted versus its fluoride concentration, and the resulting best fit line was determined using Microsoft Excel. All experimental sample fluoride peak areas were fitted to this line to measure fluoride concentration. For the ACT mouthrinse samples, a Student's t-test was used to compare the measured fluoride concentration in unused mouthrinse to the concentration reported by Johnson and Johnson. The amount of fluoride received by a subject using the mouthrinse was calculated as the amount of fluoride in an aliquot of mouthrinse minus the amount of fluoride in the expectorated samples.

Results

Ion-exchange HPLC was used to analyze the fluoride concentrations of used and unused mouthrinse. Three samples of unused mouthrinse were found to have an average fluoride ion concentration of 271.3 ppm, with a standard deviation of 9.0 ppm; the measurement's 95% confidence interval was 271.3 ± 22.3 ppm. Based on a t-test, this measured concentration of fluoride was significantly different from the concentration reported by Johnson and Johnson of 226 ppm (p<0.05). The difference between the two concentration values represented a relative error of 20% (Table I).
Table I: Measurements of fluoride ion concentration in unused mouthrinse and their propagated errors.

<table>
<thead>
<tr>
<th>Trial</th>
<th>F⁻ concentration</th>
<th>Relative Error</th>
<th>Propagated Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>272.6 ppm</td>
<td>21%</td>
<td>20.0 ppm</td>
</tr>
<tr>
<td>2</td>
<td>261.7 ppm</td>
<td>16%</td>
<td>19.3 ppm</td>
</tr>
<tr>
<td>3</td>
<td>279.5 ppm</td>
<td>24%</td>
<td>20.4 ppm</td>
</tr>
</tbody>
</table>

Mean ± SD 271.3 ± 9.0 ppm 20%

The measurement of fluoride concentration in mouthrinse samples used and then discharged by the subject allowed the determination of fluoride mass introduced into the subject’s body. Three samples of expectorated mouthrinse were found to have an average fluoride concentration of 52.4 ppm, with a standard deviation of 2.6 ppm; this measurement’s 95% confidence interval was 52.4 ± 6.4 ppm. These measurements of fluoride concentration had an average propagated error of 16.3 ppm. Based on the volume and concentration of the expectorated samples, their total fluoride content was, on average, 1.3 mg, with a 0.1 mg standard deviation. The unused mouthrinse samples had an average total fluoride content of 1.9 ± 0.1 mg. The mass fluoride introduced to the body, defined as the difference between the mass fluoride in the unused mouthrinse and in the expectorated mouthrinse samples, was, on average, 0.6 ± 0.1 mg (Table II).

Table II: Fluoride ion masses and concentrations in used and unused mouthrinse samples, and the calculated mass fluoride introduced to the body.

<table>
<thead>
<tr>
<th>Trial</th>
<th>Unused Mouthrinse</th>
<th>Expectorated Mouthrinse</th>
<th>F⁻ introduced to body</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F⁻ concentration</td>
<td>F⁻ concentration</td>
<td>F⁻ total mass ± propagated error</td>
</tr>
<tr>
<td></td>
<td>F⁻ total mass</td>
<td>F⁻ total mass</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>272.6 ppm</td>
<td>53.4 ppm</td>
<td>0.6 ± 0.4 mg</td>
</tr>
<tr>
<td></td>
<td>1.9 mg</td>
<td>1.3 mg</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>261.7 ppm</td>
<td>49.4 ppm</td>
<td>0.6 ± 0.4 mg</td>
</tr>
<tr>
<td></td>
<td>1.8 mg</td>
<td>1.2 mg</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>279.5 ppm</td>
<td>54.4 ppm</td>
<td>0.5 ± 0.4 mg</td>
</tr>
<tr>
<td></td>
<td>1.9 mg</td>
<td>1.4 mg</td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>271.3 ± 9.0 ppm</td>
<td>52.4 ± 2.6 ppm</td>
<td>0.6 mg</td>
</tr>
<tr>
<td></td>
<td>1.9 mg</td>
<td>1.3 mg</td>
<td></td>
</tr>
</tbody>
</table>
Determination of fluoride concentrations was based on a calibration curve relating HPLC chromatograms to fluoride concentrations (Figure 1). The curve, a plot of the fluoride peak areas of various fluoride standard solutions versus the solutions' fluoride concentrations, showed a linear relationship that was approximated by the equation:

$$\text{Peak Area} = (1.00 \times 10^5 \pm 6.4 \times 10^3)[F^-] + (1.3 \times 10^6 \pm 9 \times 10^5)$$

where the concentration of fluoride is measured in ppm. This linear best fit line had an $R^2$ value of 0.984.

![Calibration Curve](image)

**Figure 1: Linear best fit of fluoride peak area versus fluoride concentration.**

Based on the chromatograms of the six standards used to create the calibration curve, fluoride was detected an average of 2.07 minutes after the sample was loaded, with a standard deviation of 0.04 minutes. Furthermore, the limit of fluoride detection, defined as a fluoride peak signal to noise ratio of three to one, was found to be $0.39 \pm 0.02$ ppb.
Finally, chromatograms of mouthrinse showed anion peaks other than fluoride (Figure 2). One of these peaks, with a retention time of 3.7 ± 0.3 minutes, was identified as chloride.

![Ion Chromatogram of unused ACT mouthrinse.](image)

**Figure 2:** Ion Chromatogram of unused ACT mouthrinse.

**Discussion**

The primary objective of this experiment—to evaluate ion-exchange HPLC as a method for measuring fluoride ion concentration in mouthrinse—was achieved: HPLC was deemed inappropriate for fluoride analysis in this setting. Mean fluoride ion content and standard deviation was measured at 271.3 ± 9.0 ppm. The mean fluoride ion intake associated with the act of using mouthrinse was calculated at 0.6 mg ± 0.1 mg. Measurements of fluoride concentration in unused mouthrinse had poor accuracy, with a relative error of 20%, but good precision, with a relative standard deviation of 3%. Measurements of expectorated mouthrinse also showed good precision, with a relative
standard deviation of 5% despite the many variables involved in rinsing and expectorating the samples.

The observed variance was probably due to both chromatogram peak overlap and factors inherent in the experiment’s design. Measurements of mouthrinse, which contained several anions in addition to fluoride, varied because the peak area could not be easily measured. Peaks appeared near the fluoride peak such that the trailing edge of the fluoride peak did not reach baseline conductivity before another anion peak began; peak integration software, therefore, had to estimate the fluoride peak’s contribution to this region of peak overlap. The amount of overlap, as well as the software’s estimation of the width of the fluoride peak, varied between measurements, which would have introduced imprecision into measurements of both used and unused mouthrinse. In addition, the nature of the experiment itself introduced variance: it was difficult for the subject to spit out an equal amount of mouthrinse for each of the three trials, and this inconsistency would have caused imprecision in the measurements of expectorated sample. These sources of variance, however, were not substantial, and this method of fluoride analysis was easily precise enough for the present purposes.

Though this experiment’s measurements were reasonably precise, they were unacceptably inaccurate. A number of observations implicated the calibration curve as the primary source of inaccuracy. The low $R^2$ value (0.984) of the curve’s linear best fit line indicated that the linear approximation used in the determination of fluoride concentrations poorly approximated the actual curve. In fact, excepting the data point at 256 ppm, the points seem to exhibit a logarithmic, not a linear, relationship. The use of a linear fit to
approximate a logarithmic curve would have rendered fluoride concentration measurements inaccurate.

The fact that the relationship between chromatogram peak area and fluoride concentration is possibly logarithmic at the concentrations measured in this experiment suggested that these concentrations (from 10 to 280 ppm) were higher than the range of concentrations at which peak area is linearly related to concentration. At low concentrations of fluoride, both $F^-$ and $Na^+$ ions emerge from the first HPLC column, and then $Na^+$ ions are bound in the suppressor column. This is accomplished by the replacement of $H^+$ by $Na^+$ at ionic functional groups on the suppressor column's resin. Because $H^+$ is introduced into the mobile phase during suppression, the acid HF can form. This acid is strong enough, however, to dissociate essentially completely, so all fluoride is present as $F^-$ ion and can be detected by the conductivity detector. This process is confounded at high fluoride concentrations, because as more fluoride is introduced to the columns, more HF can form in the suppressor column. As more HF forms, the pH of the eluent lessens, and a substantial amount of HF does not dissociate. Every fluoride atom that passes the conductivity detector as part of an uncharged species, like HF, is not detected by the conductivity detector, resulting in an underestimation of fluoride concentration at high concentration. This phenomenon probably occurred in this experiment, as evidenced by the fact that the linear calibration curve fit, which did not account for the fact that peak areas become underestimates of fluoride concentration at high concentrations, produced significant overestimations of fluoride concentration in unused mouthrinse.

The fact that the fluoride peaks observed in many chromatograms used for the calibration curve had tailing ends further suggested that fluoride was measured at an
unreasonably high concentration, because tails are indicative of analyte overloading. When overloaded, the relationship between an analyte's concentration and its peak area becomes nonlinear, thus rendering a linear fit of the calibration curve, as preformed in this experiment, inaccurate.

Though the primary source of this experiment's inaccuracy was probably the inability of a linear fit to approximate the calibration curve, other factors likely contributed to the inaccuracy as well. For example, the HPLC conditions used could have introduced inaccuracy. A mobile phase flow rate of 1.5 mL per minute was chosen for this experiment, but the ion exchange column used supports lower rates. High flow rates can increase peak broadening by two mechanisms: first, high flow rate increases variance in the path length that individual ions take through the column, and second, high flow rate lessens the uniformity of anion penetrance into the ion exchange resin. In both cases, peak width is increased because individual ions take different amounts of time to travel through the column. The fact that the fluoride peak observed in mouthrinse overlapped with at least one other anion peak suggested that flow rate had not been optimized for anion separation. The overlapping peaks introduced error into fluoride concentration measurements because they artificially increased the fluoride peak area, leading to concentration over-approximation. It is difficult to determine how influential the HPLC conditions themselves were in effecting poor separation, because fluoride is notoriously hard to separate from other anions by ion exchange HPLC.

Based on these results, ion exchange HPLC is not nearly accurate enough for use in measuring fluoride concentrations in mouthrinse. In addition, the present results did not indicate a range of fluoride concentrations at which ion exchange HPLC would be an acceptably accurate analytical technique, because the range at which fluoride peak area
relates linearly to concentration was not found. Indicators of sample overloading, however, revealed that the linear range is probably far below the concentrations measured in this experiment.

The calibration curve's nonlinearity could be remedied by initially diluting all of the samples to fluoride concentrations low enough to be in the linear range. It is unclear, however, precisely where the linear range lies, so it would be prudent to investigate alternative methods for the measurement of fluoride concentration that have proven ranges of linear or logarithmic fit. One such alternative is the use of an ion-selective fluoride combination electrode. This electrode uses a single-junction, sleeve-type reference electrode to measure changes in potential related to the concentration of fluoride ion. These changes in voltage are directly related to the log of fluoride concentration, as described by the Nernst equation. Fluoride concentration can be measured in any solution with a constant, high ionic strength, provided that cations that can bind F⁻ are sequestered by the addition of cyclohexylenedinitrilotetraacetic acid. This technique has been shown to measure fluoride concentrations from 1-20 ppm with high accuracy (0.5% relative error) and precision, so it could be used in this experiment provided that all samples are diluted to fluoride concentrations within this range⁹.

Conclusion

Fluoride ion content in commercial mouthrinse was determined by ion exchange chromatography. Mean fluoride ion content with standard deviation was measured at 271.3 ± 9.0 ppm. The mean mass of fluoride transferred to the body by the use of mouthrinse was calculated at 0.6 mg ± 0.1 mg. Measurements of fluoride ion concentration in unused
mouthrinse had poor accuracy, with a relative error of 20%, but good precision, with a relative standard deviation of 3%. The experimental precision and reproducibility were very encouraging. In contrast, the large relative errors suggested that ion exchange chromatography is unsatisfactory in producing accurate results. Closer analysis of the calibration curve, however, revealed that samples were simply too concentrated with fluoride ions.

The results of this experiment did not prove ion exchange chromatography to be an inadequate tool for measuring fluoride ion concentration in commercial mouthrinse. Rather, the experiment indicated that at high ion concentrations, there is no longer a linear relationship between peak area and concentration. When fluoride is present above this concentration range, measurements become inaccurate. Thus, in future ion exchange chromatography experiments, samples must be diluted to concentrations low enough for linear approximation. To ensure accurate linear or logarithmic fit of the calibration curve in a future experiment, an alternative analytical technique with a proven range of logarithmic fit, such as an ion-selective fluoride electrode, should be considered. If HPLC is to be used again, a range of fluoride concentrations that gives a linear calibration curve must be established, and the mobile phase linear flow rate should be altered to maximize peak separation. The use of more standard solutions for the calibration curve should also noticeably increase the experiment's accuracy.

References

(2) Health Advisory for Fluoride in Groundwater.

(3) Skoog; West; Holler; Crouch; "High Performance Liquid Chromatography."


