

# **Ion Chromatography Validation for the Analysis of Anions in Gunshot Residue**

**Student Report Written for:**

**Dr. Samide**

**CH 424**

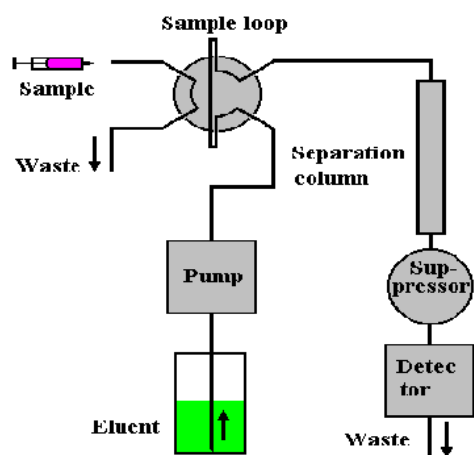
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## **Abstract**

The purpose of this study is to determine whether or not ion chromatography can be used to match gunshot residue back to the bullet manufacturer, which would be helpful because the actual bullet casing is not always available at crime scenes. In this report, the validation of an ion chromatography method for seven different anions (chloride, fluoride, bromide, nitrite, nitrate, sulfate, and phosphate) is described. Calibration curves were generated for each anion and a standard reference material was examined to determine the accuracy of this method. Spike recoveries were also performed and limits of detection and quantitation were determined. It was found that the calibration curves showed excellent linearity at higher concentrations (75 ppb to 2.5 ppm). None of the standard reference calculations were statistically similar to the known value according to a one sample T-test because the standard deviations were so small; however, the percent similarities between the standard reference calculations and the known value all fell within an acceptable range of 80-120%.

## Introduction

Ion chromatography (IC) separates ions on the basis of attractions between the charged analyte and the oppositely charged stationary phase (Harris 589). The analyte passes through a degasser, followed by a pulse dampener, a guard column, and the analytical column. After the analytical separation occurs, the eluent passes through the suppressor, which is full of  $H^+$  ions that diffuse into the eluent and neutralize ions in the mobile phase, because a neutral solution is necessary to measure the signal that is produced by the conductivity of the analyte ions. The concentration of analyte can then be determined from the area under the peak that forms when each ion elutes.



**Figure 1: Diagram of IC** (Metrohm Ion Analysis)

Ion chromatography has few applications in forensic science, but it has several applications in the analysis of anions. Singh, Kumar, Sen, and Majumdar used the IC to determine whether fluoride waste could be quantitatively removed from spent pot liner, which is a hazardous waste produced at cell houses of aluminum smelters. The study showed that fluoride concentrations of about 10 ppm could be successfully reduced to below the 2 ppm regulatory limit. They also found that 100% extraction could be achieved with fluoride concentrations of 10

ppm in the presence of other ions, such as iron, aluminum, calcium and cyanide, and that the IC was most effective with fluoride concentrations below 10 ppm. Furthermore, an optimum pH range of 7 to 10 and a flow rate of 4 mL/min were demonstrated to be the most effective in removing fluoride from the column (Sing, et. al, 1999).

In a validation study done by Jackson and co-workers, the linear ranges of fluoride, nitrite, nitrate, bromide, and phosphate were found to be 0.1 to 100 ppm, and the linear ranges of chloride and sulfate were 0.2 to 200 ppm. Detection limits ranged from 1.7 to 9.6 ppb for all anions. Method reproducibility was also verified, with 0.5% and 0.2% RSD values for peak area and retention times. After validating these seven ions, they extended their analysis to perchlorate and found that it could be quantitatively removed from wastewater, in part due to its low detection limit of 0.5 ppb.

When studying ion fluxes between tomato plants and simulated acid mists, Foster used the IC to analyze nitrates, sulfates, and phosphates in aqueous samples prepared from tomato leaf samples. This method was selected because of its high precision (all %RSD's were below 0.6% from aqueous samples and below 1.5% from foliar samples). High accuracy also proved to be an advantage to this method, and the method was validated using certified water and foliar standards from the National Bureau of Standards (Foster, 1990).

An article studying barley seedlings found that the IC was a very effective way to analyze sulfate, phosphate, nitrate, and nitrite ions. Employing the IC to determine the plastidic concentration of anions from chloroplast extractions, they found that there are higher concentrations of sulfites and sulfates in barley seedlings located in urban settings where there is significant sulfur dioxide pollution. The IC was chosen because of its high level of reproducibility (Wolfenden and Wellburn, 1986).

According to Hatsis and Lucy, who studied column coatings to increase the speed of analysis, iodate and phosphate had very high detection limits in comparison to chloride, nitrite, bromide, nitrate, and sulfate. Use of the IC was chosen because of its low detection limits (all anions studied had detection limits in the parts per billion range). Furthermore, they found the IC to be a precise method because they calculated a 0.4% RSD for peak area and a 0.7% RSD for retention time (Hatsis and Lucy, 2003).

A study by Johns and co-workers used the IC to determine whether ions from explosive residue could be distinguished from ions present in the environment. They analyzed 18 anions present in explosives: acetate, benzoate, bromate, carbonate, chlorate, chloride, chlorite, chromate, cyanate, fluoride, formate, nitrate, nitrite, perchlorate, phosphate, sulfate, thiocyanate, and thiosulfate. Detection limits for all anions ranged from 2.0 to 27.4 ppb. This method was selected due to its high level of reproducibility and precision, with % RSD's ranging from 0.01 to .22% for retention times and 0.29 to 2.16% for peak area. The results were confirmed by doing a similar analysis on the same samples with capillary electrophoresis (Johns, 2008).

In this experiment, the IC will be validated using seven anions: chloride, phosphate, fluoride, bromide, sulfate, nitrate, and nitrite. Gunshot residue will be collected from cloth targets and brought into solution. Anions that may be found in gunshot residue, either in high concentrations or in trace amounts, will then be analyzed using the calibration curves generated in this validation report. The purpose is to determine whether or not gunshot residue can be matched to the bullet manufacturer on the basis of differing ratios of these anions. It will be difficult to obtain high concentrations of gunshot residue, so this method has been selected due to its low detection limits and high precision. There is little forensic research concerning anions, so

this method will help determine if gunshot residue can be matched to the bullet manufacturer according to the ratio of anions present in the residue.

## Materials and Methods

### *Materials*

High Purity Standards (1000 ppm—fluoride, chloride, sulfate, nitrate, nitrite, phosphate, and bromide) were used to make standards. Fluka Certified Multianion Standard Solution, containing 10.0 mg/kg  $\pm 0.2\%$  of F, Cl, Br, NO<sub>3</sub>, SO<sub>4</sub>, and PO<sub>4</sub>, was used as a standard reference material. Deionized water was produced in-house by first treating tap water by reverse osmosis and then passing the RO water through three ion-exchange sediment beds. DI water was used to make all solutions and canal water was used in the spike recovery analysis.

### *Instrumentation*

All IC analyses were carried out using a Metrohm 881 Compact IC pro system equipped with a Metrosep A Supp 5 150/4.6 mm column operating at a flow rate of 0.7 mL/min and a column temperature of 30 °C. The mobile phase consisted of a Supp 5 Eluent Concentrate 0.32 M Na<sub>2</sub>CO<sub>3</sub>/0.10 M NaHCO<sub>3</sub> and elution times were reproducible as shown in Table 1.

**Table 1: IC retention times ( $\pm 5.0\%$ ) in minutes.**

Ion	Retention times
Fluoride	4.065
Chloride	5.782
Nitrite	6.723
Bromide	8.322
Nitrate	9.37
Phosphate	12.728
Sulfate	14.867

### *Calibration Curves*

In the first trial, seven anions, chloride, phosphate, fluoride, bromide, sulfate, nitrate, and nitrite, were analyzed using the IC to generate calibration curves. 19 standards, ranging from 5.0 ppb to 5.0 ppm were prepared in 25 mL volumetric flasks using a 10 ppm stock solution of the seven anions. After reviewing this data and determining an approximate linear range, 18 new standards were prepared, this time ranging from 75 ppb to 2.5 ppm. A stock of 2.5 ppm was used in making these solutions. A standard reference material containing 0.4 ppm of fluoride, chloride, bromine, nitrate and phosphate was also prepared and analyzed using the IC. Linear regressions were performed on the standards and used in analysis of the standard reference material. T-tests were performed to determine if the standard reference values determined from the linear regressions were the same as the 0.4 ppm that was analyzed. A percent similarity was also calculated to determine accuracy.

### *Spike Recoveries*

Canal water was also analyzed using the same method so that a spike recovery analysis could be done. Water was collected from the canal and filtered once through a 0.45 micron filter pad and once through a 0.22 micron filter pad. Three sets of spikes and samples were run. The first was prepared by adding canal water (200.0  $\mu$ L) into each of the two volumetric flasks, one of which was spiked with the 10 ppm stock of chloride, phosphate, fluoride, bromide, sulfate, nitrate, and nitrite (200.0  $\mu$ L). The second set was prepared using canal water (500.0  $\mu$ L) in each volumetric and again spiked with the 10 ppm stock (200.0  $\mu$ L). The third set was prepared using canal water (1.0 mL) and a spike of 10 ppm stock (200.0  $\mu$ L). Different amounts of canal water were used because some anions, such as chloride, were very highly concentrated, so a very small amount of canal water was needed in order for its signal to fit within the calibration range.

However, the diluted sample necessary for chloride analysis was not concentrated enough in fluorides or nitrates, so it was necessary to use greater amounts of canal water. All samples were analyzed using the IC. The linear regressions from the second set of standards were used in the spike recovery analysis.

## Results and Discussion

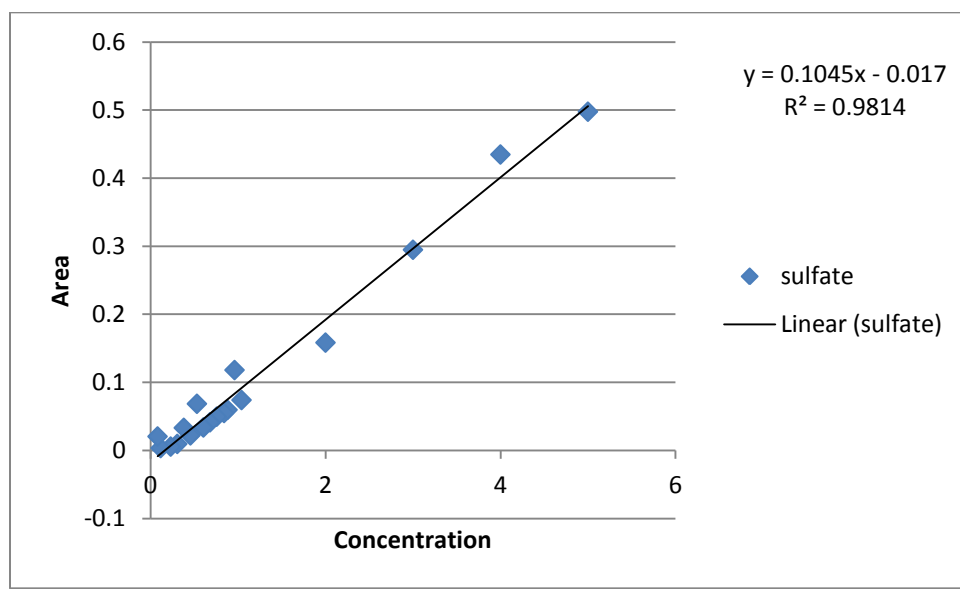


Figure 2: Sulfate calibration curve from the first trial (5 ppb to 2.5 ppm)

Figure 2 shows one of the seven calibration plots that were generated from the first set of standards. It appears that the sulfate plot is nearing the end of its linear range because the upper portion of the graph starts to curve downwards. This same trend was seen in the other six plots as well. There is also a lot of scatter in the lower concentrations, showing that the graph may be nearing the end of its lower linear range as well. Furthermore, the  $R^2$  value of 0.9814 is lower than it should be. Because of this, the next set of standards was prepared with concentrations between 75 ppb to 2.5 ppm to stay within the linear range of all seven anions.

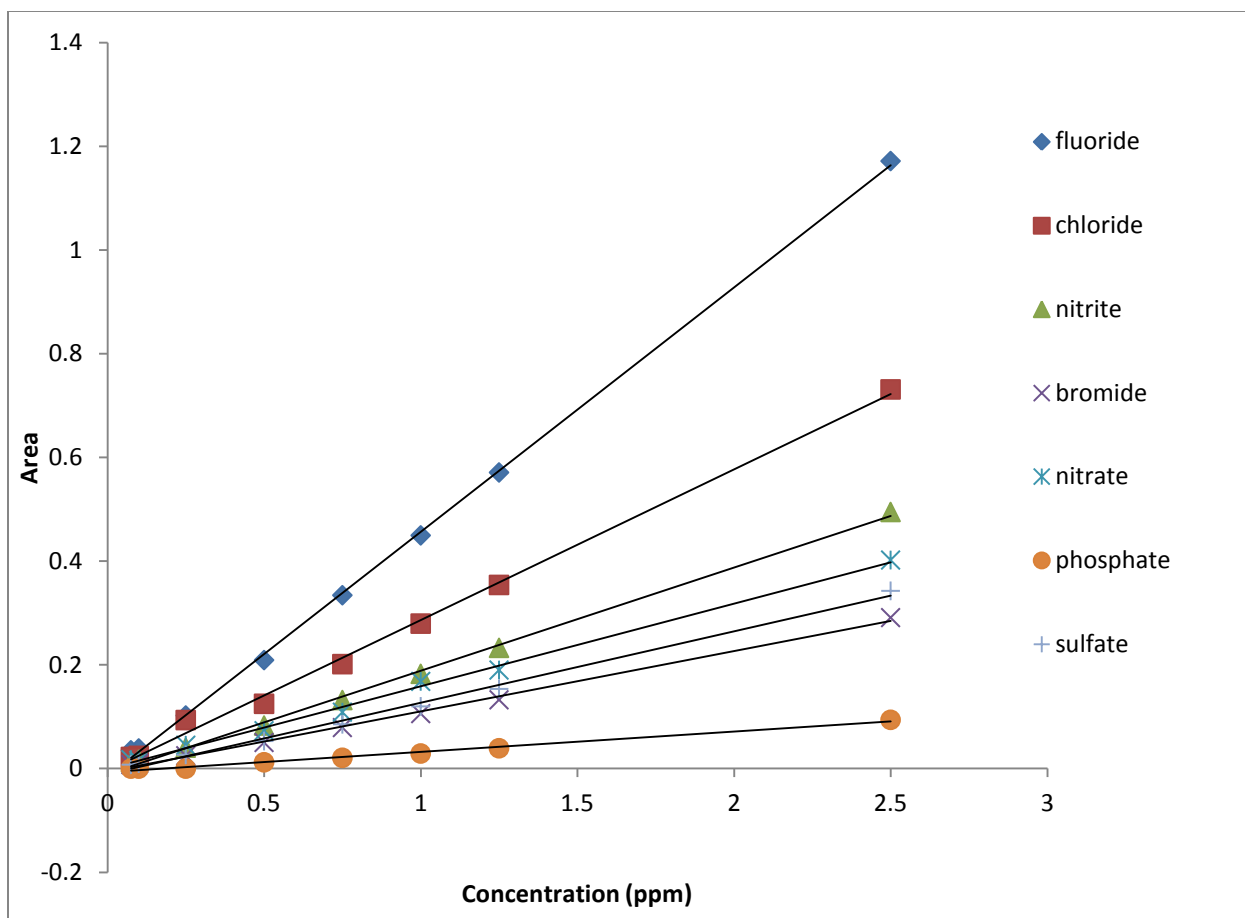


Figure 3: Adjusted calibration curves generated from linear range data in trial 1 (75 ppb to 2.5 ppm).

Table 2: Linear regressions and R<sup>2</sup> values from Figure 4.

Calibration Curves		
	Linear Regression	R <sup>2</sup> value
Fluoride	$y = 0.4713x - 0.0145$	0.9995
Chloride	$y = 0.2906x - 0.0044$	0.9968
Nitrite	$y = 0.199x - 0.0104$	0.9982
Bromide	$y = 0.1163x - 0.0057$	0.9974
Nitrate	$y = 0.1594x - 0.0004$	0.9967
Phosphate	$y = 0.1379x - 0.0109$	0.991
Sulfate	$y = 0.0392x - 0.0071$	0.9956

The calibration curves in the second trial showed much more linearity than in the first trial (as can be seen from higher R<sup>2</sup> values). Furthermore, all of the % RSD's were well below 5%, showing that this method is very precise. There is also a wide range of sensitivities in the 7 anions analyzed. The IC is most sensitive to fluoride, followed by chloride, nitrite, nitrate,



sulfate, bromide, and phosphate. Higher sensitivities are more accurate because it shows that the instrument is more sensitive to smaller changes in analyte.

**Table 3: Limits of detection and quantitation in ppb.**

<b>Ion</b>	<b>Limit of Detection</b>	<b>Limit of Quantitation</b>
Fluoride	4.0	13.78
Chloride	0.654	2.17
Nitrite	1.095	3.751
Bromide	1.86	6.2
Nitrate	4.83	16.1
Phosphate	6.1	253.9
Sulfate	4.7	15.74

According to Table 3, all of the limits of quantitation, with the exception of phosphate, fell below 75 ppb, which is the lowest concentration used in the calibration plot in Figure 3. This means that all data generated within our calibrated range can be quantified, with the exception of phosphate, which can only be detected. This could be due to low sensitivities or to high standard deviations.

**Table 4: Concentrations determined from the standard reference material using Figure 4. A percent difference of the calculated concentrations from the known value of 0.4 ppb is shown in column 2. P-values from a one sample T test are shown as well.**

<b>Ion</b>	<b>Concentration</b>	<b>Percent Similarity</b>	<b>P-value</b>
Fluoride	0.36226	90.57%	1.30E-06
Chloride	0.391373	97.84%	1.77E-04
Sulfate	0.409234	102.31%	0.031981
Nitrate	0.464846	116.21%	3.11E-05
Bromide	0.361135	90.28%	4.41E-18
Phosphate	0.42602	106.51%	0.008493

As can be seen in Table 4, when the calculated concentrations were compared to the true value of 0.4 ppm, a one sample T-test showed them to be significantly different. T-tests were performed at an alpha level of 0.05. The P-values show the probability that the difference between the two values observed was due merely to natural sampling variation. Since all of the P-values are below 0.05, the differences observed between the true value and the calculated

values are statistically significant. However, this is likely due to the fact that the standard deviations were so low. Since the precision is so high, it is more difficult to achieve high accuracy as well, according to the T-test. This is because in the equation for the T-value, the standard deviation is in the denominator, meaning that a very small standard deviation will lead to a very high T-value, which leads to a rejection of the null hypothesis. Thus, a percent similarity was calculated to compare the actual values without taking precisions into account. According to the percent similarity study, all of the values fall within an acceptably accurate range of 80%-120%. Even though the T-test shows the calculated concentrations to be inaccurate, the percent similarities show them to be acceptably accurate.

**Table 5: Spike recoveries performed on canal water.**

Ion	Amounts used	Spike recovery
Fluoride	1 mL canal water +200 µL spike	89.73%
Chloride	200 µL canal water +200 µL spike	105.24%
Nitrate	1 mL canal water +200 µL spike	99.07%
Sulfate	500 µL canal water + 200 µL spike	85.21%

Spike recoveries were performed on filtered canal water to further determine the accuracy of the method. Different concentrations of sample were used so that all four ions found in the canal water would be of the right concentration to fit within their calibration curve. Ideally, the spike of a given ion should be close to the concentration of that ion in the sample so that the differences in concentration between the spiked and unspiked samples can be detected. Table 5 shows that the IC is accurate in the analysis of fluoride, chloride, nitrate, and sulfate because all of them had spike recoveries that fell within the acceptable range of 80%-120%.

In conclusion, the method used in this study seems to be both accurate and precise. Furthermore, the  $R^2$  values that were generated from the high range calibration plots are very high (all above 0.99). This means that at least 99% of the changes in signal depend on changes in

concentration. Both the spike recovery analysis and the percent similarity calculated from a comparison of calculated concentrations to the known concentration show that this method is accurate because all values were between 80-120%. Because all %RSD's generated from the IC data were below 5%, it can also be concluded that this method is precise.

On the basis of this validation study, gunshot residue samples can now be analyzed for fluoride, chloride, bromide, nitrate, nitrite, phosphorous, and sulfur using the IC. The calibration plots shown in Figure 3 will be used to determine the concentration of ions in the residue, and the ratio of ions present in the residue will be compared to the ratio of ions present in the bullet shell casing. In future studies, it may also be valuable to look at other anions that could be present in gunshot residue, such as perchlorates and chlorates. It would also be helpful to collect gunshot residue from the shooter's hand and to determine whether or not that could be matched to the bullet manufacturer.

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## **Appendix**

Determination of concentration from calibration curve of the equation  $y=mx+b$ :

concentration= $(\text{area}+b)/m$  where  $m$  is the slope and  $b$  is the  $y$  intercept

Spike recovery analysis: % recovery= $(\text{concentration of spiked sample}-\text{concentration of unspiked sample})/\text{amount of spike added}$

Limit of detection= $(3*SD)/m$  where  $m$  is the slope of the calibration plot and the standard deviation used is the lowest calculated standard deviation for a given ion.

Limit of quantitation= $(10*SD)/m$

% difference=  $\text{calculated concentration}/\text{known concentration of } 0.4 \text{ ppm}$