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# Extraction, Cleanup, and Analysis of the Perchlorate Anion in Tissue Samples

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There have been increasing human health and ecological concerns about ionic perchlorate ($ClO_4^-$) since it was detected in drinking water sources in 1997 (Urbansky 1998). Perchlorate is known to affect normal thyroid function, causing subsequent hormone disruption and potential perturbations of metabolic activities (Saito et al. 1983). Evaluating tissue levels and identifying possible sources of perchlorate contamination are critical in determining significant toxicological and ecological exposure.

Recent advances in analytical capabilities have allowed for improved detection of the perchlorate anion in water (Dionex 1998). Utilization of these improved analytical capabilities has produced data suggesting more widespread contamination of ground and surface water with perchlorate, especially in areas of the western U.S. (Urbansky 1998). In contrast to the wealth of human health data, little data exist on potential ecological effects of environmental perchlorate contamination. Fish and wildlife are also likely to be chronically exposed to perchlorate in areas where such contamination exists. Because thyroid hormones play homologous roles in fish and wildlife as they do in humans, there exists the potential for disruption of endocrine homeostasis in these species as well (Smith et al. 2001).

The unique characteristics of perchlorate (soluble anion) make accurate quantitation in biological matrices difficult. The presence of additional ions, proteins, lipids, and other biomolecules that can clog or foul ion exchange columns further confounds accurate determination of perchlorate concentrations in biological tissues and fluids. Although a method for detecting perchlorate in plant tissues has been developed (Ellington and Evans 2000), to our knowledge there are no methods for determining perchlorate concentrations in animal tissues, especially those exposed to perchlorate outside of the laboratory environment. The ability to detect perchlorate in exposed animals is critical to the risk assessment process.

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Since the primary difficulty in perchlorate analysis of tissue extracts via ion chromatography is related to high background conductivity, we evaluated a variety of potential cleanup methods for tissue extracts. Our primary objective was to determine the usefulness of these cleanup methods in reducing background conductivity while maintaining the detectability of low (ppb) perchlorate concentrations in tissue extracts. The method described here was successfully used to determine perchlorate in ecological receptors inhabiting a perchlorate-contaminated site in East Texas (Smith et al. 2001).

## MATERIALS AND METHODS

All perchlorate calibration standards were prepared from a 100 µg/mL certified sodium perchlorate standard (AccuStandard, Inc., New Haven, CT) in distilled, deionized water (18MΩ). Sodium hydroxide (eluent) was purchased from Fisher Scientific and diluted with distilled, deionized, water (18MΩ) to the appropriate concentration (100 mM) for the ion chromatography (IC).

Bovine kidney samples were obtained from the Texas Tech University Meat Laboratory. In our preliminary methods development, we determined (via IC) that kidney tissue extracts as opposed to liver or thyroid were the most problematic as far as background signal. Therefore, we focused our efforts on developing cleanup methods for this matrix. The cortex of the kidney was sectioned into pieces weighing approximately 4 g each. Kidney samples were placed inside glass beakers and each (with the exception of the control samples) was perfused with 1 mL of a sodium perchlorate solution of known concentration. Perfusion was performed using a 3-mL syringe with a 25-gage needle. Multiple areas of each tissue sample were perfused. All glass beakers were covered with Parafilm® and stored (5° C) for one week. After one week, kidney samples were dried for 1 h at 65° C. Beakers were rinsed with distilled, deionized, water (18MΩ) and the rinsates were analyzed for perchlorate as described below to calculate a mass balance of perchlorate.

Dried tissue samples (weighed) were extracted using an Accelerated Solvent Extractor (ASE 200, Dionex Corp.). Samples were placed inside 22-mL stainless steel extraction cells, and extracted using the following procedure. Cells were heated for 5 minutes at 100° C, filled with distilled, deionized, water (18MΩ), and pressurized to 1500 psi. Total extraction time was 15 minutes. At the completion of the extraction procedure, extract volume was recorded.

Initial evaluations of potential cleanup procedures focused on the use of solid phase extraction (SPE) cartridges for reducing background conductivity of tissue extracts. Ten different types of SPE columns were used to test cleanup efficiency: strong cation exchange (SCX), carboxylic acid (CA), strong anion exchange (SAX), weak anion exchange ($NH_2$), silica (Si), Alumina (Al), florisil (F), octadecyl ($C_{18}$), methyl ($CH_3$), and octyl ($C_8$). Depending on the nature of the sorbent, SPE cartridges were conditioned as appropriate prior to use. One mL of

the tissue extract was diluted with 4 mL of distilled, deionized, water (18MΩ) and added to the various SPE columns. Column eluate was filtered (Acrodisc®, 0.45 μm) and analyzed as described below.

We also evaluated the use of ion exchange technology as a potential cleanup procedure for reducing background conductivity of tissue extracts. Similar technology is used in ion chromatography for suppressing eluent signal. Our evaluation was focused on Nafion® membranes. Nafion® is a registered trademark for Dupont's brand of perfluorinated ion exchange polymer that contains small proportions of sulfonic or carboxylic ionic functional groups. Nafion® has excellent chemical stability (similar to Teflon®) while at the same time allowing ion transport.

A 10-cm section of Nafion® tubing (total length 15 cm, 0.5 mm i.d.) was submerged in 1 mM $H_2SO_4$. One mL of tissue extract (spiked with perchlorate) was eluted through the Nafion® tubing (approximately 0.5 mL /min) and collected. Following the tissue extract, 4 mL of distilled, deionized, water (18MΩ) was eluted through the tubing and collected in the same container as the tissue extract eluate. Cleaned extracts were filtered (Acrodisc®, 0.45 μm) and analyzed by ion chromatography as described below.

In order to determine the reproducibility of the cleanup procedure, we extracted a blank kidney sample (no perchlorate) and spiked the extract with a known amount of sodium perchlorate. The spiked extract was used to evaluate variability and potential losses of perchlorate in the cleanup procedure. Distilled, deionized, water (18MΩ) spiked with perchlorate was used as a control (clean) extract to determine possible matrix effects of the extract on cleanup efficiency. Eluates from the cleanup procedure were filtered (Acrodisc®, 0.45 μm) and analyzed as described below.

Eleven kidney samples were perfused (procedure described above) with perchlorate to create 3 different perchlorate tissue concentrations: low (0.9 μg perchlorate/g tissue), medium (5.2 μg perchlorate/g tissue), and high (10.3 μg perchlorate/g tissue). Samples were extracted using ASE and extracts were cleaned using Nafion®. Purified extracts were analyzed as described below.

Analysis of perchlorate ion was carried out using a Dionex DX-500 Ion Chromatography System equipped with a GP50 gradient pump, a CD20 conductivity detector, and an AS40 automated sampler (Dionex Corp.). PeakNet® chromatography software was used to control the system. Ion separation was made with a Dionex IonPac AS16 (250 mm x 4.0 mm) analytical column. Conditions for the system were as follows: analysis time = 12:00 min; flow rate = 1.0 mL/min; eluent = 100 mM sodium hydroxide; injection volume = 1000 μL. Ion detection was by suppressed conductivity in the external water mode. A seven-point standard curve was constructed from constant volume injections of calibration standards of 1.0, 2.5, 5, 10, 20, 100, and 500 ppb

(ng/mL). Computer-generated peak areas were used to measure sample concentrations in an external standard mode. Using the analytical method described above, the quantitation limit for perchlorate anion in distilled water was 1.0 ppb (ng/mL).

## RESULTS AND DISCUSSION

The extraction efficiency of perchlorate from kidney was initially tested at sufficiently high perchlorate concentrations such that cleanup of the extracts was not necessary in order to detect perchlorate against the background signal. Results of these tests indicated that perchlorate extraction efficiency was nearly quantitative (> 90%). These results also indicated that the evaluation of possible cleanup methods using perchlorate-spiked extracts could be conducted without the confounding effects of extraction efficiency.

Evaluations of potential SPE cleanup procedures focused on reducing background conductivity of tissue extracts. Initial results indicated that SAX, $NH_2$, $CH_3$, CA, and F were not effective at reducing background signal and/or produced poor recoveries of perchlorate. Cleanup efficiencies were improved using $C_8$ and $C_{18}$, however, these phases are likely to only remove organic-based compounds which might interfere with the analysis. It is unlikely that these phases removed any ions contributing to the background signal. Perchlorate recoveries were 71% ± 6% and 50% ± 6% for $C_8$ and $C_{18}$, respectively.

Cleanup efficiencies based on perchlorate recovery were improved dramatically using the more polar sorbents: Al and Si, and the ion exchange sorbent SCX and ion exchange membrane, Nafion® (**Table 1**). Nearly quantitative recovery of perchlorate (≥ 85%) was observed for all of these sorbents with Si providing the

**Table 1.** Cleanup efficiency of polar and ion exchange sorbents used for kidney extracts treated with perchlorate.

| Phase | Expected Conc. (ppb) | Measured Conc. (ppb) | % Difference (measured vs. expected) |
|---|---|---|---|
| ALL* | 26 | 26.30 ± 0.52 (n = 12) | 1 |
| Al | 10 | 11.16 ± 0.52 | 12 |
| Si | 10 | 10.41 ± 0.17 | 4 |
| SCX | 10 | 10.00 ± 0.58 | 0 |
| Nafion® | 200 | 169 ± 27 | 16 |

*Mean recovery (all phases) for perchlorate spiked in distilled, deionized, water.

best precision and SCX providing the best accuracy. A recent paper by Ellington and Evans (2000) also identified Al as an effective sorbent for cleanup of plant extracts containing perchlorate.

The sorbents and ion exchange membrane were also effective in reducing background signal (conductivity). SCX, Si, and Nafion® were the most effective; Column eluates had signals similar to perchlorate in distilled, deionized, water and were less than the signal from an uncleaned tissue extract (**Figure 1**). While

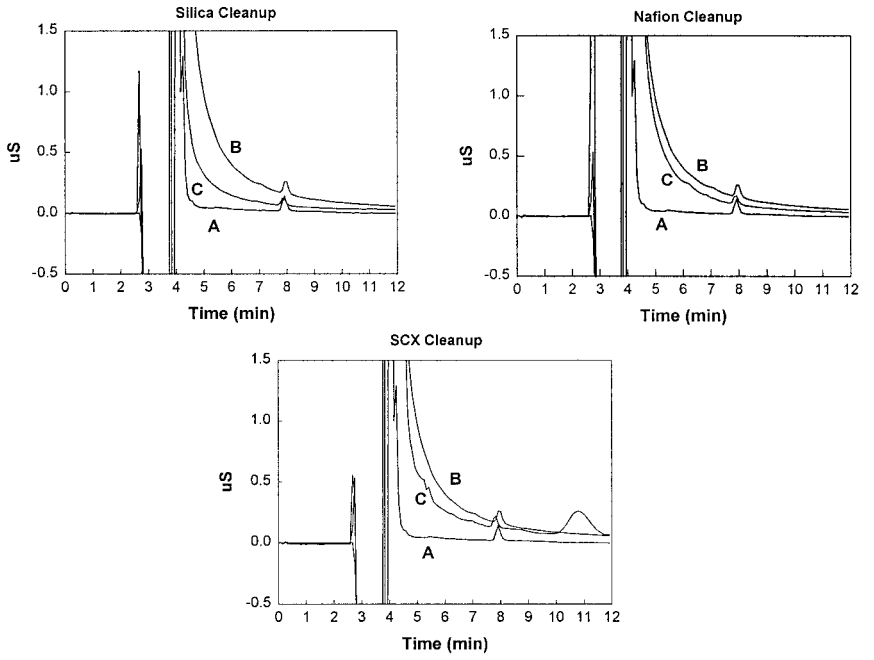


**Figure 1.** Ion chromatograms showing the effect of various cleanup technologies: silica, Nafion®, and strong cation exchange (SCX). In all chromatograms, the concentration of perchlorate was 10 ppb. Chromatogram A is 10 ppb perchlorate in distilled, deionized, water. Chromatogram B is a 1:100 dilution of a perchlorate-spiked kidney extract. Chromatogram C is a 1:100 dilution of a perchlorate-spiked kidney extract after cleanup.

Si cleanup resulted in the greatest reduction in signal, Nafion® had the highest capacity for cleanup. In other words, Nafion® was capable of cleaning extracts larger than 1 mL. While the other sorbents (SCX, Si, Al) were effective on small extract volumes (< 1mL), their capacity was exceeded when larger samples were

used. In addition, the effectiveness of SCX cleanup is very dependent on proper conditioning of the sorbent. We conditioned the SCX cartridges sequentially with distilled water, 3 mM HCl, and distilled water. The last step is critical as chloride ion remaining on the sorbent will increase background signal of the sample eluate.

The results of perchlorate recovery experiments (ASE extraction/Nafion® cleanup) are presented in **Table 2**. In these tests, kidney samples were perfused

**Table 2.** Perchlorate recovery from kidney tissue.

| Sample | Tissue Weight (g) | Expected Conc. (ppb) | Measured Conc. (ppb) | % Recovery |
|---|---|---|---|---|
| L1 | 2.00 | 105 | 67.2 | 64 |
| L2 | 2.15 | 105 | 52.7 | 50 |
| L3 | 2.49 | 105 | 67.1 | 64 |
| L4 | 2.10 | 105 | 60.6 | 58 |
| M1 | 2.05 | 526 | 314 | 60 |
| M2 | 2.06 | 526 | 427 | 81 |
| M3 | 1.75 | 526 | 360 | 68 |
| H1 | 1.93 | 1053 | 682 | 65 |
| H2 | 1.77 | 1053 | 684 | 65 |
| H3 | 2.13 | 1053 | 733 | 70 |
| H4 | 1.88 | 1053 | 658 | 62 |

Samples were extracted using ASE. Extracts were cleaned using Nafion®. Overall perchlorate recovery was 64% ± 8%.

with perchlorate to produce 3 perchlorate tissue concentrations (0.9 ppm, 5.2 ppm, and 10.3 ppm). Perchlorate recovery did not vary with tissue concentration. Overall perchlorate recovery was 64% ± 8%. This mean recovery is somewhat less than perchlorate recovery (85%) from the spiked extracts after cleanup with Nafion®, but appears to be consistent among the replicates. It is also important to note that the capacity of Nafion® is greater than the other sorbents we tested. The ability to use Nafion® on larger extract volumes while still maintaining background signal reduction will be critical in improving the perchlorate detection limits in tissues. Currently, based on the typical tissue sample size used in our method development (2 g), the limit of detection is 180 ng perchlorate/g tissue.

Recently, we have tested (1) other extraction solvents as alternatives to water, and (2) other SPE cartridges, in order to improve the removal of interfering compounds to perchlorate analysis. For example, ethanol extractions appear to be useful in precipitation of protein in samples such as blood. In addition,

OnGuard™ cartridges (Ag, Ba, and H) from Dionex appear to also be useful in extract cleanup.

Using commercially available cleanup technology, we developed a method for the cleanup of tissue extracts to be analyzed for perchlorate. The cleanup method reduces the high background conductivity typical of most tissue extracts while maintaining decent levels of perchlorate detection. Our goal in developing the method was to be able to quantify potential perchlorate exposure to wild animals. We have used this method to evaluate tissue concentrations of perchlorate from both laboratory and field tests (**Table 3**). Some of the field evaluations included a

**Table 3.** Concentration of perchlorate found in tissues of animals collected from a perchlorate-contaminated site.

| Sample | Tissue Concentration (ppb) |
|---|---|
| *Sigmodon hispidus* (kidney) | 1300 |
| Unidentified bird (viscera) | 7190 |
| *Peromyscus gossypinus* (liver composite) | 1870 |
| Unidentified bird (viscera) | 900 |
| Unidentified bird (viscera) | 4750 |

number of potential receptor organisms inhabiting a site in east Texas where certain areas are contaminated with ammonium perchlorate (Smith et al. 2001). To our knowledge, the use of the extraction and cleanup method described herein produced the first incidence of perchlorate exposure among wild animals reported in the scientific literature.

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