Determination of Perchlorate in Vegetation Samples Using Accelerated Solvent Extraction and Ion Chromatography

Bruce Richter and Brett Murphy
Thermo Fisher Scientific, Salt Lake City, UT, USA

Application Note 356

Introduction

Perchlorate (ClO_4^-) is an environmental contaminant that has been found in drinking, ground, and surface waters in several states within the United States. Most of the contaminated sites have been traceable to sources near military installations or manufacturing sites where perchlorate salts are used to manufacture rocket propellant, munitions, or fireworks. The solubility, mobility, and persistence of perchlorate have resulted in the contamination of drinking water, soil, and vegetation in several areas.

Perchlorate has been shown to present a health-based risk to humans.[1] Exposure to perchlorate disrupts uptake of iodide by the thyroid gland. For this reason, the EPA has placed this anion on its Contaminant Candidate List for drinking water. The EPA has not established any enforceable health regulations for perchlorate in drinking water or related matrices. Nevertheless, states such as California and Massachusetts have set individual action levels restricting the amount of perchlorate in drinking water.

Many scientists have shown that plants grown with perchlorate tainted water become contaminated with perchlorate.[2] The determination of perchlorate in water at the low part per billion (ppb) level can be challenging, however, sample preparation for water samples is generally not considered extremely difficult. The sample preparation necessary to measure perchlorate levels in vegetation is much more challenging and tedious. Analytical protocols for perchlorate typically begin with some type of liquid-solid extraction. High-speed blending and ultrasonication extraction are the most common methods of removing perchlorate from soil or vegetation samples. These methods are labor intensive, yet simple and easy to use, but are not efficient enough to extract tightly bound ions such as perchlorate from complex vegetation or other biosolid matrices. Additionally, these techniques often require post-extraction cleanup steps such as Solid-Phase Extraction (SPE) using different absorbents. Accelerated solvent extraction has been shown to overcome complex analyte-matrix interactions and was successfully applied to the extraction of perchlorate from several matrices. In addition to automating the extraction procedure, the accelerated solvent extraction technique coupled with Thermo Scientific™ Dionex™ OnGuard™ resins produces a clean extract that can be directly injected into an ion chromatograph.

Accelerated solvent extraction extracts solid samples rapidly using minimal amounts of solvent. A typical 5 g sample of soil or plant material would require approximately 10 to 100 times the weight of the sample in water. Compared to other manual based extraction methods, accelerated solvent extraction also provides a significant reduction in time and labor. Accelerated solvent extractions are typically complete in about 10 to 15 min. Recoveries and precision (RSD) are comparably better than blending or sonication techniques. Furthermore, accelerated solvent extraction can be completely automated and provide in-cell cleanup to remove potential interferences.

Thermo SCIENTIFIC

With the accelerated solvent extraction technique, solvent is pumped through the sample from top to bottom in a stainless steel extraction chamber. After solvent is introduced, the sample is heated. Pressure is used to maintain the solvent as a liquid. Typical accelerated solvent extraction temperatures range from 80 °C to 120 °C, depending on the sample, with a maximum temperature of 200 °C. Accelerated solvent extraction uniquely combines dynamic and static extraction methods, resulting in an efficient extraction in a relatively short period of time. At the end of an accelerated solvent extraction method, a solvent flush followed by a gas purge separates the solvent and analytes from the sample. Because elevated extraction temperatures are used in accelerated solvent extraction, analyte diffusion rates are accelerated compared to soaking, sonication, or blending extraction methods. Higher temperatures also act to overcome the enthalpy associated with adsorption of the analytes onto sites at the matrix surface or the intracell or interstitial spaces of vegetation material.

This application note provides the details of using accelerated solvent extraction for the determination of perchlorate in soil, milk, and several plant matrices. The method provides a rapid means of extracting perchlorate from all of the aforementioned matrices using only water as an extraction solvent. The benefits of this method are simplicity, speed of analysis, and automation. Accelerated solvent extraction allows the rapid extraction and in-line cleanup of a large number of samples with minimal labor. Accelerated solvent extraction technology allows automated, uninterrupted extractions of up to 24 samples for the Thermo Scientific Dionex ASE™ 200 Accelerated Solvent Extractor System (sample sizes less than 3 g) and twelve samples for the ASE 300 Accelerated Solvent Extractor System (sample sizes greater than 3 g). Computer control of all extraction parameters is available for both instruments.

Equipment

- Dionex ASE 200 or ASE 300 Accelerated Solvent Extractor System
  - 60 mL collection vials (P/N 048784)
  - 250 mL collection bottles (P/N 056284)
  - Glass fiber filters (P/N 047017 for ASE 200, P/N 056781 for ASE 300)
  - Dionex OnGuard II Sample Pretreatment Cartridges
    - Ag (P/N 057089)
    - Ba (P/N 057093)
    - H (P/N 057083)
    - RP (P/N 057083)
  - Dionex ASE Prep DE (P/N 062819)
  - Analytical balance with 0.1 mg resolution
- Thermo Scientific Dionex ICS-2500 Chromatography System:
  - GP50 Gradient Pump with vacuum degas option
  - EG50 Eluent Generator with Thermo Scientific EluGen™ EGC II NaOH cartridge (P/N 058908)
  - AS40 Autosampler
  - LC30 Chromatography Oven
  - CD25 Conductivity Detector with conductivity cell
  - Thermo Scientific Dionex Chromeleon™ 6.6 Chromatography Data Systems Software (Service Pack 3)

Chromatographic Conditions

Columns:	Thermo Scientific Dionex IonPac™ AS16 Analytical, 2 × 250 mm Dionex IonPac AG16 Guard, 2 × 50 mm Dionex IonPac Cryptand C1 Concentrator, 4 × 35 mm
Eluent:	0.50, 65, and 100 mM NaOH
Flow Rate:	0.25 mL/min
Temperature:	35 °C
Backpressure:	2300 psi
Detection:	Suppressed conductivity, Thermo Scientific Dionex ASRS™ ULTRA II Suppressor, external water mode, 100 mA current
Run Time:	46 min

Accelerated Solvent Extraction Conditions for Perchlorate

Extraction Solvent:	Water
Pressure:	1500 psi
Temperature:	80 °C
Equilibration Time:	5 min
Extraction Time:	5 min (static)
Solvent Flush:	30% (of cell volume)
Nitrogen Purge:	120 s (after extraction)
Extraction Cycles:	3
Cell Sizes:	33 mL and 100 mL

Accelerated Solvent Extraction Sample Preparation

Due to the large amount of matrix interferences seen in the initial work done with alfalfa, it was decided to incorporate the use of Dionex OnGuard H (Hydronium), Ag (Silver), Ba (Barium), and RP (Poly-divinylbenzene) pretreatment cartridges into the extraction cells. These cartridges contain ion-exchange resins, which remove alkali earth metals, halides, sulfates, and hydrophobic compounds from the sample. It was suspected that large amounts of chloride and sulfate ions were seen in the initial extractions of alfalfa and spinach, hence the need for ion-exchange resins. Basic alumina was also added to the extraction cell. The use of the Dionex OnGuard cartridge resins along with the basic alumina greatly reduced the amount of interferences detected in the resulting extracts. In these experiments, the cartridges are opened and the resins are scooped out into the extraction cells. The chromatograms shown in Figure 1 compare accelerated solvent extraction alfalfa extracts obtained using no in-line cleanup (green) and Dionex OnGuard resins combined with basic alumina (blue) in the Dionex ASE Extractor extraction cell.

Prior to extraction, the 100 mL cells are prepared from bottom to top as follows: two GFB filters, 3.0 g of Dionex OnGuard H, a GFB filter, 6.0 g of OnGuard Ag, a GFB filter, 3.0 g OnGuard Barium, a GFB filter, 18 g basic alumina, a GFB filter, 1.8 g OnGuard RP, a glass fiber filter, and then fill the remainder of the cell with Dionex ASE Prep DE. The 33 mL cells are prepared in the same manner with proportionally less of each resin.

Prior to analysis, each of the extracts was filtered using a 0.2 µm polyethersulfonate syringe filter.

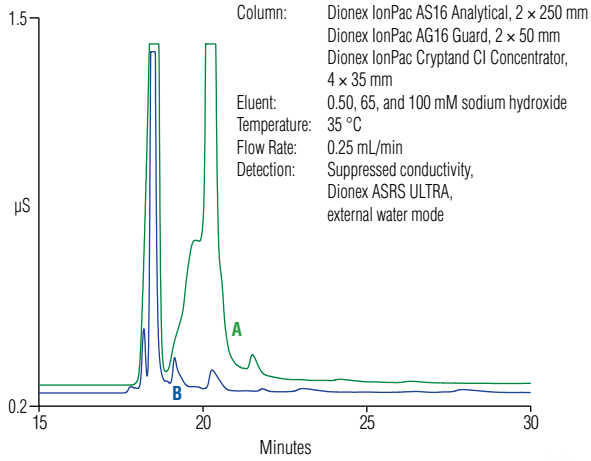


Figure 1. Alfalfa extracts obtained using (A) no in-line cleanup and (B) Dionex OnGuard resins combined with basic alumina in the Dionex ASE Extractor extraction cell.

Preparation of Solutions and Reagents

Reagents and Standards

Deionized water (DI H_2O), Type I reagent grade, 18 Ω-cm resistance or better

Sodium perchlorate, 98% A.C.S reagent grade or better

A.C.S reagent grade sodium salts

Sodium hydroxide (NaOH) 50% w/w

Stock Perchlorate Standard Solution

Dissolve 0.3078 g of sodium perchlorate in 250 mL of deionized water for a 1000 mg/L standard solution. This stock standard is stable for at least one month when stored at 4 °C.

Stock Synthetic Sample Matrix Stock Solution

Dissolve 8.6 g of sodium bicarbonate, 9.3 g of sodium sulfate, and 10 g of sodium chloride in 250 mL of deionized water for a 25.0 g/L stock solution. One mL of this Laboratory Synthetic Sample Matrix Stock Solution (LSSMSS) is then added to all calibration standards. Next, 62.5 mL of the LSSMSS is diluted to 250 mL to give a solution with a concentration of 6.25 g/L. The resulting solution (Laboratory Synthetic Sample Matrix Fortification Solution, LSSMFS) is added to all field samples to give a final concentration of 100 mg/L for the sodium compounds.

Working Standard Solutions

Prepare working standards at lower concentrations by diluting the appropriate volumes of the 1000 mg/L stock standard with deionized water. These working standards are prepared at 10.0 mg/L and 1.0 mg/L. Dilutions of these standards are then used to prepare the calibration standards. Calibration standards were prepared at 1, 2, 5, 10, 25, 50, and 100 µg/L for the initial work, and then at 5, 10, 20, 50, 100, and 200 µg/L for the replicate studies of corn, melon, and spinach. One mL of the LSSMSS is also added to each calibration standard. The calculated correlation coefficient for one of the calibration curves used for analysis of the vegetation extracts was 0.9986.

System Preparation and Setup

Samples

Milk, melon, spinach, alfalfa, and corn samples were obtained from a local grocery store. The soil was purchased from Wibby Environmental (Golden, CO, USA). Representative samples (5 g) were placed into a mortar with 10 g of Dionex ASE Prep DE (cleaned as detailed above), ground with a pestle, and then added to the Dionex ASE Extractor extraction cell. The mixture was then spiked with the appropriate amount of perchlorate standard. The cells were allowed to stand overnight at 4 °C. The final volume of each of the resulting extracts was then adjusted to either 40 mL (if the 33 mL Dionex ASE Extractor cells were used) or 100 mL (if 100 mL Dionex ASE Extractor cells were used).

It was also possible to eliminate part of the so-called matrix effect associated with plant or fruit matrices with the Dionex AS40 autosampler and Dionex IonPac Cryptand preconcentration column. Two sample vials are prepared for use with the Dionex AS40 autosampler. One contains 2 mL of sample that had been spiked with the LSSMFS and the second contains 1 mL of 10 mM sodium hydroxide. The sodium ion from the LSSMFS reacts with the Dionex IonPac Cryptand column to retain perchlorate. As the sodium ion concentration increases, the capacity of the Dionex IonPac Cryptand column to retain perchlorate also increases. The sodium hydroxide solution washes away any contaminants from the preconcentration column. The perchlorate is then eluted onto the analytical column for analysis. A schematic of this system configuration is shown in Figure 2.

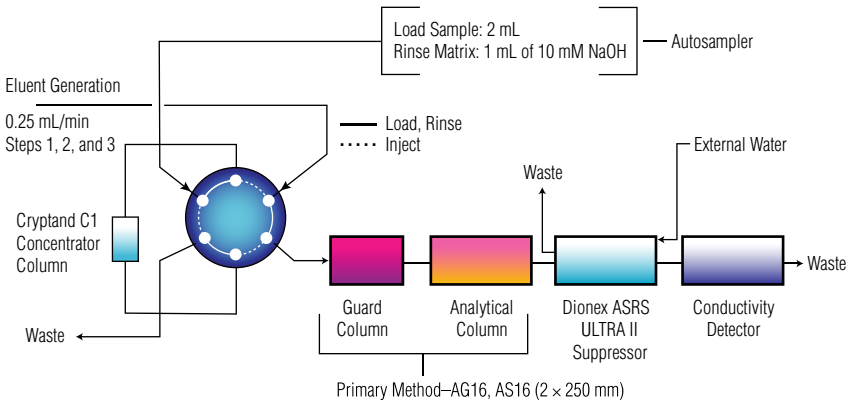
Results and Discussion

Initial accelerated solvent extraction studies were performed with soil, alfalfa, corn, and milk. The samples were prepared as described above in the "Experimental" section. Each sample matrix was extracted in replicates of five. The recovery data and reproducibilities for each set of extractions are shown in Table 1.

Table 1. Recovery data for accelerated solvent extraction of perchlorate.

Analyte	Perchlorate (ppb)	%Recovery*	%RSD
Soil	50	106	7.89
Alfalfa	50	94.2	8.24
Corn	50	88.7	8.86
Milk	25	118.7	1.57

*Analysis was performed using EPA Method 314.1 with a Dionex ICS-2500 ion chromatography system.



NaOH Eluent Generation

Step	Function	Conc.	Time
1	Perchlorate Transfer	0.5 mM	12 min
2	Analysis	65 mM	16 min
3	Column Cleanup	100 mM	2 min

Figure 2. Schematic of the system configuration for the extraction of perchlorate in vegetable samples.

Figure 3 compares chromatograms of a 3 g soil sample that had been spiked with perchlorate and extracted with water. The resulting perchlorate concentration is 50 ppb (ng/g). The chromatogram of a soil "blank" extract is overlaid with the spiked sample to show that there are no interferences with the perchlorate peak.

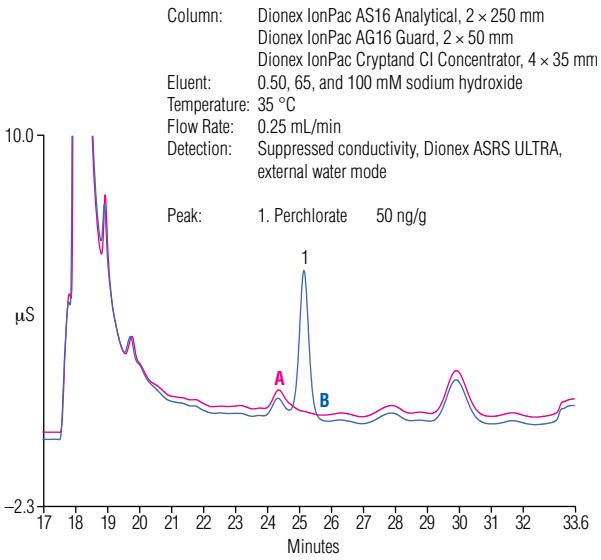


Figure 3. Chromatograms of (A) a soil "blank" obtained using accelerated solvent extraction, and (B) a 3 g soil sample spiked with perchlorate and extracted with water.

Figure 4 shows the chromatogram resulting from an accelerated solvent extract of a 5-g melon sample spiked with perchlorate. The resulting perchlorate concentration is 10 ppb (ng/g). The chromatogram of a "blank" melon extract is overlaid with the spiked sample to show that there are no extraneous peaks in the "blank" extract that interfere with the perchlorate peak.

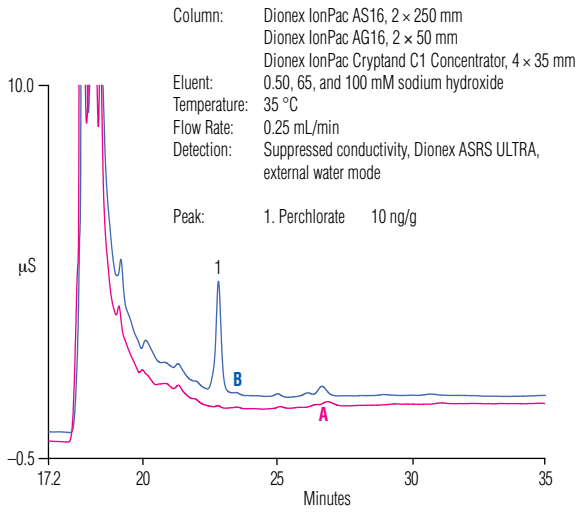


Figure 4. Chromatograms of (A) a melon "blank" obtained using accelerated solvent extraction, and (B) a 5 g melon sample spiked with perchlorate.

Figure 5 shows the chromatogram of a 5 g spinach sample spiked with perchlorate. The resulting perchlorate concentration is also 10 ppb (ng/g). The chromatogram of a spinach "blank" extract is again overlaid with the spiked sample to show that there are no interferences with the perchlorate peak.

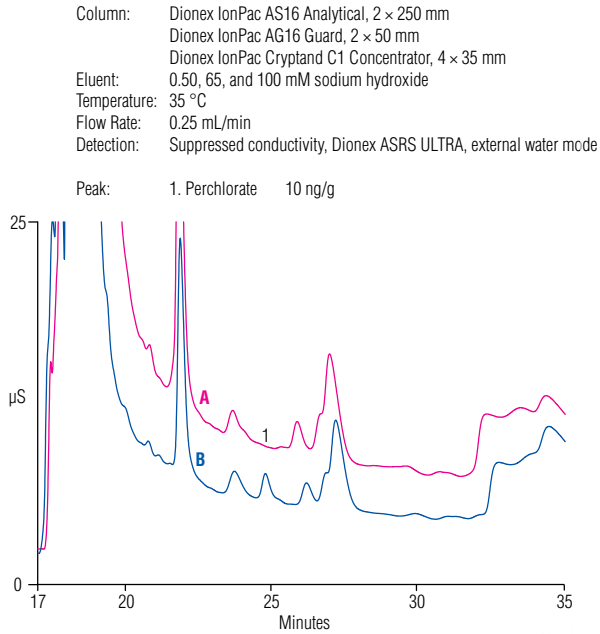


Figure 5. Chromatograms of (A) a spinach "blank" obtained using accelerated solvent extraction, and (B) a 5 g spinach sample spiked with perchlorate.

As a result of the experiments summarized in Table 1, we decided to continue the recovery studies at a lower spike level. A more detailed study was done for corn, melon, and spinach. Each sample matrix was spiked at three different levels of perchlorate (10, 50, and 200 ppb) and the extractions were done in replicates of seven. The results from these experiments are summarized in Table 2.

Table 2. Recovery and reproducibility data for accelerted solvent extraction of perchlorate.

Matrix	Perchlorate (ppb)	%Recovery*	%RSD
Melon	10	110	2.48
	50	96.8	2.54
	200	103	5.51
Corn	10	102	5.36
	50	88.7	8.86
	200	95.7	6.80
Spinach	10	106	5.40
	50	101	7.17
	200	97.9	6.53

*Analysis was performed using EPA Method 314.1 with a Dionex ICS-2500 ion chromatography system.

Figure 6 shows a graph summarizing and comparing the data obtained from the study. There appears to be no matrix or concentration effect under the conditions tested.

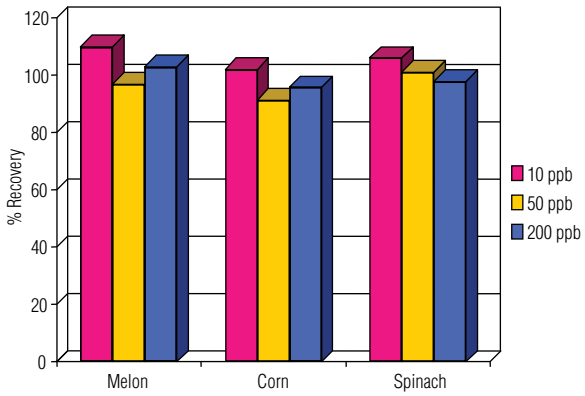


Figure 6. Bar graph summarizing the percent recovery data of perchlorate extracted from spiked samples of melon, corn, and spinach using accelerated solvent extraction.

The method performance of the method was also evaluated by calculating the method detection limit (MDL). This was done by multiplying the standard deviation of the seven replicates of the low-level samples by 3.143 (as per EPA guidelines). The reliable quantization limit (RQL) was calculated by multiplying the MDL by 4. Table 3 summarizes these results.

Table 3. Summary of the method performance.

Matrix	Avg. Recovery (%, n = 21)	*MDL (µg/kg)	*RQL (µg/kg)
Melon	103.3	0.72	2.9
Corn	96.3	1.4	5.6
Spinach	101.6	2.0	8.0

*Analysis was performed using EPA Method 314.1 with a Dionex ICS-2500 ion chromatography system.

Conclusion

The accelerated solvent extraction method detailed in this application note provides a fast and efficient extraction of perchlorate from various food and soil samples. The extracted samples can be analyzed directly using IC coupled with a conductivity detector. As can be seen from the recovery and reproduci-bility data mentioned above, the results from the accelerated solvent extraction are very similar, if not better than the popular ultrasonication methods. Using accelerated solvent extraction saves time, solvent, and labor when compared to manual extraction techniques. This also demonstrates that it is possible to achieve ppb level of detection from vegetation samples with very little sample cleanup prior to analysis.

References

1. Q. Cheng, F. Liu, J. E. Canas, and T. A. Anderson. *Talanta*, **2005**, in press.
2. W. A. Jackson, P. Joseph, P. Laxman, K. Tan, P. N. Smith, L. Yu, T. A. Anderson. *J. Agric. Food Chem.* **2005**, *53*, 369.
3. *An Improved Method for Determining Sub-ppb Perchlorate in Drinking Water Using Preconcentration/ Matrix Elimination Ion Chromatography with Suppressed Conductivity Detection by U.S. EPA Method 314.1*. AN 176, in press, Dionex Corporation (now part of Thermo Scientific), Sunnyvale, CA.
4. Method 314.1, U.S. Environmental Protection Agency, Cincinnati, OH, **2005**.

Australia +61 3 9757 4486
Austria +43 1 333 50 34 0
Belgium +32 53 73 42 41
Brazil +55 11 3731 5140
China +852 2428 3282
Denmark +45 70 23 62 60
France +33 1 60 92 48 00
Germany +49 6126 991 0
India +91 22 2764 2735
Italy +39 02 51 62 1267
Japan +81 6 6885 1213
Korea +82 2 3420 8600
Netherlands +31 76 579 55 55
Singapore +65 6289 1190
Sweden +46 8 473 3380
Switzerland +41 62 205 9966
Taiwan +886 2 8751 6655
UK/Ireland +44 1442 233555
USA and Canada +847 295 7500

Thermo SCIENTIFIC
Part of Thermo Fisher Scientific

AN70397_E 12/12S