

Determination of Perchlorate in Vegetation Samples Using Accelerated Solvent Extraction (ASE[®]) and Ion Chromatography

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 (CCL) 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 (ASE) 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 ASE technique coupled with Dionex OnGuard[®] resins produces a clean extract that can be directly injected into an ion chromatograph.

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ASE 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, ASE also provides a significant reduction in time and labor. ASE extractions are typically complete in about 10 to 15 min. Recoveries and precision (RSD) are comparably better than blending or sonication techniques. Furthermore, ASE can be completely automated and provide in-cell cleanup to remove potential interferences.

With the ASE 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 ASE extraction temperatures range from 80 °C to 120 °C, depending on the sample, with a maximum temperature of 200 °C. ASE uniquely combines dynamic and static extraction methods, resulting in an efficient extraction in a relatively short period of time. At the end of an ASE method, a solvent flush followed by a gas purge separates the solvent and analytes from the sample. Because elevated extraction temperatures are used in ASE, 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 ASE 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. ASE allows the rapid extraction and in-line cleanup of a large number of samples with minimal labor. ASE technology allows automated, uninterrupted extractions of up to 24 samples for the ASE 200 (sample sizes less than 3 g) and twelve samples for the ASE 300 (sample sizes greater than 3 g). Computer control of all extraction parameters is available for both instruments.

EQUIPMENT

ASE 200 or ASE 300 system

60-mL collection vials (Dionex P/N 048784)

250-mL collection bottles (Dionex P/N 056284)

Glass fiber filters (P/N 047017 for ASE 200, P/N 056781 for ASE 300)

OnGuard II Sample Pretreatment Cartridges

Ag (P/N 057089)

Ba (P/N 057093)

H (P/N 057085)

RP (P/N 057083)

ASE[®] Prep DE (P/N 062819)

Analytical balance with 0.1 mg resolution

Dionex ICS-2500 chromatography system consisting of:

GP50 Gradient Pump with vacuum degas option

EG50 Eluent Generator with EluGen[®] EGC II

NaOH cartridge (P/N 058908)

AS40 Autosampler

LC30 Chromatography Oven

CD25 Conductivity Detector with conductivity cell

Chromeleon[®] 6.6 Chromatography Management Software (Service Pack 3)

CONDITIONS

Chromatographic Conditions

Columns: IonPac[®] AS16 Analytical, 2 × 250 mm (P/N 55376)

IonPac AG16 Guard, 2 × 50 mm (P/N 055379)

IonPac Cryptand C1 Concentrator, 4 × 35 mm (P/N 062893)

Eluent: 0.50, 65, and 100 mM NaOH

Flow Rate: 0.25 mL/min

Temperature: 35 °C

Backpressure: 2300 psi

Detection: Suppressed conductivity, ASRS[®] ULTRA II, external water mode, 100 mA current

Run Time: 46 min

ASE 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

ASE 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 (Fisher Scientific — used as received) was also added to the extraction cell. The use of the 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 ASE alfalfa extracts obtained using no in-line cleanup (green) and OnGuard resins combined with basic alumina (blue) in the ASE extraction cell.

Prior to extraction, the 100-mL cells are prepared from bottom to top as follows: two GFB filters, 3.0 g of 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.

To ensure clean resins, each prepared cell was extracted under the same ASE conditions as the samples. During this step, the resins and the ASE Prep DE were cleaned of any potential interference with the perchlorate ion. Ten g of the “clean” ASE Prep DE was mixed with 5 g of sample. The resulting mixture was then ground in a mortar and pestle and added back into the cell prior to extraction.

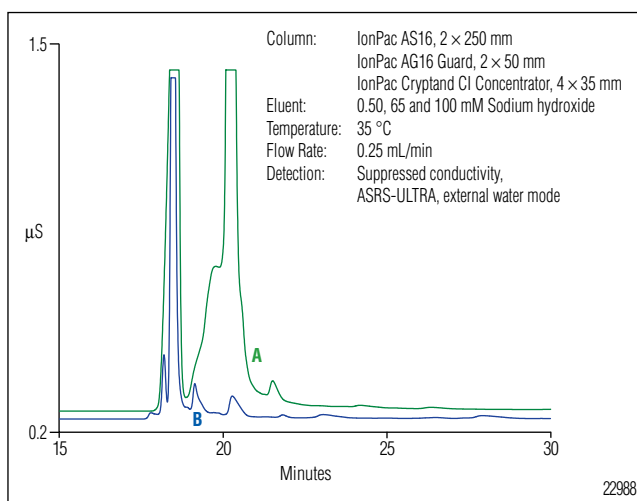


Figure 1. Alfalfa extracts obtained using (A) no in-line cleanup and (B) OnGuard resins combined with basic alumina in the ASE extraction cell.

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

PREPARATION OF SOLUTIONS AND REAGENTS

REAGENTS AND STANDARDS

Deionized water (DI H₂O), Type I reagent grade,
18 Ω-cm resistance or better

Sodium perchlorate, 98% ACS reagent grade or better
(Aldrich)

ACS reagent grade sodium salts (Mallinckrodt, Fisher)

Sodium Hydroxide (NaOH) 50% w/w (Fisher Scientific)

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). Representative samples (5 g) were placed into a mortar with 10 g of ASE Prep DE (cleaned as detailed above), ground with a pestle, and then added to the ASE 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 ASE cells were used) or 100 mL (if 100-mL ASE cells were used).

It was also possible to eliminate part of the so-called matrix effect associated with plant or fruit matrices with the AS40 Autosampler and Cryptand preconcentration column. Two sample vials are prepared for use with the AS40. 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 Cryptand column to retain perchlorate. As the sodium ion concentration increases, the capacity of the 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.

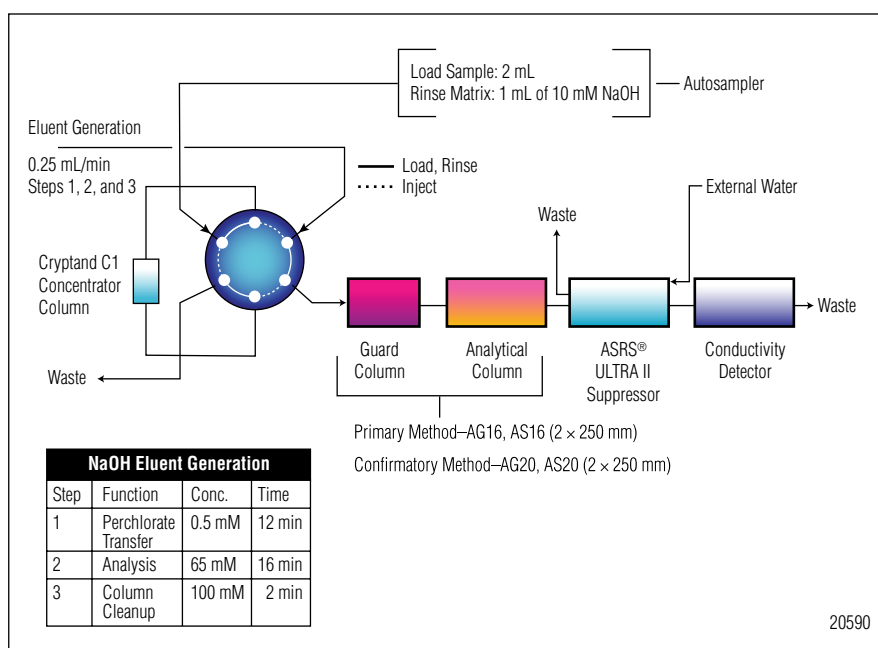


Figure 2. Schematic of the system configuration for the extraction of perchlorate in vegetable samples.

RESULTS AND DISCUSSION

Initial ASE 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 ASE Extraction of Perchlorate (n=5)

Matrix	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.

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” ASE extract is overlaid with the spiked sample to show that there are no interferences with the perchlorate peak.

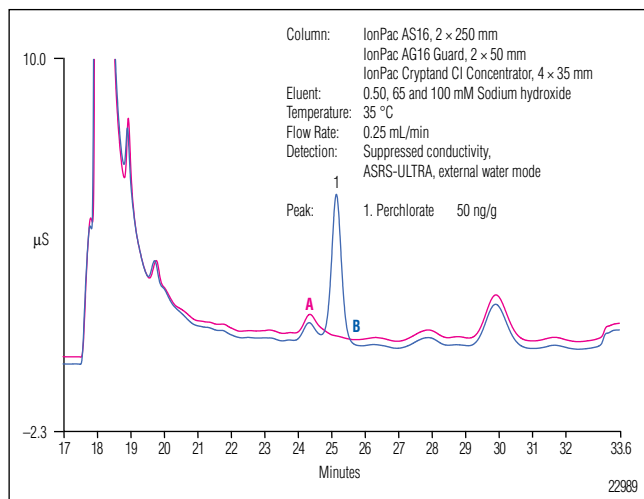


Figure 3. Chromatograms of (A) a soil “blank” obtained using ASE, and (B) a 3-g soil sample spiked with perchlorate and extracted with water.

Figure 4 shows the chromatogram resulting from an ASE 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.

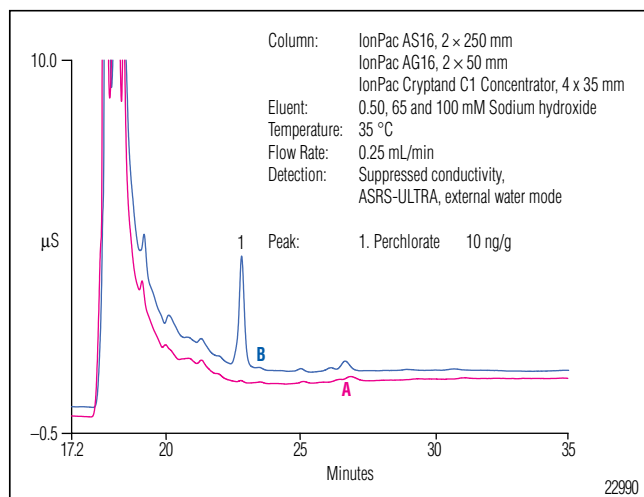


Figure 4. Chromatograms of (A) a melon “blank” obtained using ASE, and (B) a 5-g melon sample spiked with perchlorate.

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.

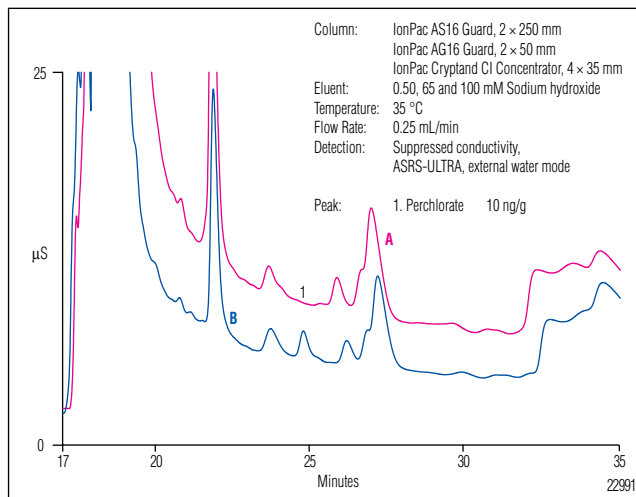


Figure 5. Chromatograms of (A) a spinach “blank” obtained using ASE, and (B) a 5-g spinach sample spiked with perchlorate.

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 ASE 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.

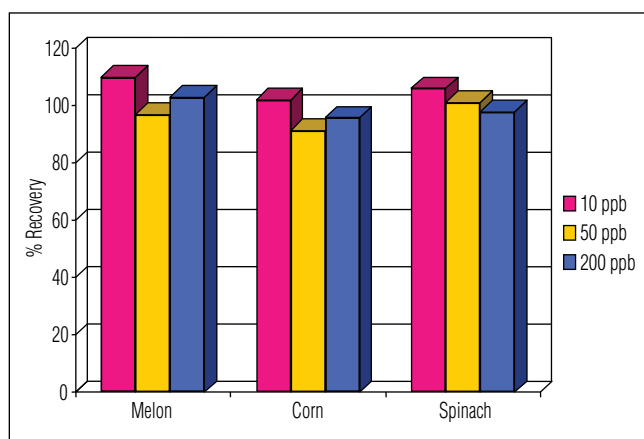


Figure 6. Bar graph summarizing the percent recovery data of perchlorate extracted from spiked samples of melon, corn, and spinach using ASE.

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 ASE 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 reproducibility data mentioned above, the results from the ASE extraction are very similar, if not better than the popular ultrasonication methods. Using ASE 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.

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LPN 1830 PDF 05/06
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