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Ion Chromatographic Determination of Perchlorate in Foods by On-Line Enrichment and Suppressed Conductivity Detection

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Systemic uptake of perchlorate anion, a rocket fuel component and potential thyroid function disruptor, by leafy vegetables and other crops grown in contaminated waters is a public health concern. A column-switching anion-exchange chromatographic method with suppressed conductivity detection, described in this paper, achieved a 3−6 μg/kg method limit of quantitation in analysis of the wet weight edible portion of cantaloupe, carrots, lettuce, and spinach samples with field-incurred perchlorate. A test portion was blended with dilute nitric acid, and the extract was filtered under vacuum. A portion of the measured filtrate was acidified to pH ~2 by addition of cation-exchange resin, 4 mL was passed through a graphitized carbon cleanup column, and an aliquot of a collected fraction was pushed through a short precolumn for anion extraction, enrichment, and injection onto the analytical column. Statistical comparison with determination by tandem mass spectrometry−ion chromatography analysis of untreated filtrate revealed that the difference between means was not significant at the 95% confidence level (P value $\geq$ 0.12) for crops tested. In addition, the method was applied to cooked vegetables processed as baby food.

KEYWORDS: Perchlorate; ion chromatography; suppressed conductivity; anion exchange; determination; food

INTRODUCTION

Perchlorate anion (ClO_4^-) competitively inhibits iodide uptake by the thyroid gland's sodium−iodide symporter (NIS) protein and, at high levels, may disrupt production of two iodine hormones [triiodothyronine (T_3) and thyroxine (T_4)] essential for metabolic activity. Thiocyanate (SCN^-) and nitrate (NO_3^-) anions are less potent competitors. When Chinese hamster ovary cells stably expressing human NIS were exposed to varying concentrations of each anion, the relative potency of ClO_4^- to inhibit $^{125}I^-$ uptake at the NIS was found to be 15, 30, and 240 times that of SCN^-, I^-, and NO_3^-, respectively, on a molar concentration basis (*1*). Recast on a mass basis, 1 μg of perchlorate was as potent as 9 μg of thiocyanate, 38 μg of iodide, or 150 μg of nitrate in vitro uptake by human NIS transfected into animal cells.

At particular risk to perchlorate exposure are fetuses and infants because neural, brain, and skeletal development depend on sufficient levels of T_3. Pregnant and nursing women transfer perchlorate by placental and mammary NIS systems, respectively. In a small study of 36 lactating women from 18 states in the United States, perchlorate levels in breast milk up to 92 μg/L and averaging 10.5 μg/L have been reported (*2*). The U.S. National Academies of Science, in reviewing studies on human health effects from perchlorate ingestion, found that a reference dose (RfD) of 0.7 μg/kg per day was protective of public health for even the most sensitive groups of people over a lifetime of exposure (*3*). In February 2005, the U.S. Environmental Protection Agency (EPA) established this finding as the official RfD for perchlorate (*4*).

Ammonium perchlorate is widely used as an oxidant/propellant in solid rocket fuels and in explosives such as fireworks and munitions. Production and military facilities have contaminated ground and surface waters through leaching, seepage, and improper disposal of material past its shelf life. Known perchlorate releases have been identified in 35 states (*5*, *6*). As an environmentally stable, strong electrolyte, perchlorate is highly mobile in a water system from a point source of entry. Perchlorate in Lake Mead and the lower Colorado River, sources of drinking water for ~15 million people in the southwestern United States, came from a production plant in Henderson, Nevada, that had been making ammonium perchlorate for four decades (*7*). Since 1999, when controls began at the plant, >3 million pounds of perchlorate have been captured, and annual average concentrations at sampling points have declined substantially (*8*). In 2004, nine monthly samples taken at a Colorado River aqueduct intake for the Metropolitan Water District (serving Los Angeles and Southern California) were below the 4 μg/kg limit of detection, a 50% decline since 2000. A portion of this water irrigates lettuce, cantaloupe, alfalfa, and other crops grown in the Imperial Valley of California and Arizona near Yuma. Studies (*9*, *10*) have shown that growing

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plants can systemically accumulate perchlorate from water. Bioconcentration factors [(μg/kg fresh weight)/(μg/L)] may reach many 100-fold, with perchlorate preferentially translocated to leaves. For example, higher concentrations were found in the outer leaves of lettuce, which are more extensively removed from iceberg than from romaine and leaf varieties before human consumption (*11*). The U.S. winter lettuce crop comes predominantly from this growing region; sampling and analysis of the edible portion of lettuce by the U.S. Food and Drug Administration (FDA) in 2004 (*12*) found perchlorate up to 129 μg/kg, although the mean ($N = 138$) was more than an order of magnitude lower at 10.5 μg/kg. Forage plants such as alfalfa may be a source of perchlorate found in dairy milk (*12*−*14*).

Perchlorate occurs naturally in salt deposits in the Atacama Desert of Chile where nitrate is mined for fertilizer. Although U.S. fertilizers are made from synthetic materials and presently do not contain perchlorate (*15*), some fertilizers acceptable for organic farming may contain potash and saltpeter from natural deposits with significant quantities of perchlorate (*16*). Oxygen isotopic abundance, which showed a large and positive ^{17}O anomaly in natural as compared to synthetic perchlorate (*17*), may help identify environmental contamination from suspected atmospheric sources, such as perchlorate in the groundwater of the southern high plains (includes the Texas Panhandle) where clear evidence of anthropogenic sources was missing (*18*).

Early analytical methods development (*19*) focused on detecting perchlorate in water to provide data for the EPA's unregulated contaminants monitoring rule program (ClO_4^- was placed on the candidate contaminant list). Sample cleanup was usually minimal despite the wide use of ion chromatography (IC) with conductivity detection (CD), which cannot confirm analyte identity. In contrast, chemically diverse matrices such as food have taken days to clean up an extract for chromatographic measurement (*10*, *11*, *13*, *20*). Generally, freeze drying the sample, a time-consuming step, was employed to concentrate the perchlorate residue before extraction and cleanup. Recently, speed, specificity, and sensitivity were achieved with IC interfaced by electrospray ionization (ESI) to a tandem mass spectrometer (MS/MS) and use of $^{18}O_4$-labeled perchlorate internal standard (IS) for analysis of food (*14*) or urine (*21*) without extract cleanup. Without the labeled IS, standard additions were necessary to mitigate groundwater matrix effects on the perchlorate signal. Flow injection (FI)-ESI-MS/MS (*22*) enabled detection to 0.5 μg/kg. Similarly, high-field asymmetric waveform ion mobility spectrometry (FAIMS), a technique that separates gas-phase ions by ion mobility in an applied electric field, was performed without liquid chromatography and with a single stage quadrupole. FI-ESI-FAIMS-MS analysis of waters (*23*) achieved μg/kg detection limits from 0.5 (river water) to 0.050 (tap water); application to food extracts has not been reported.

Methodology employing costly MS instrumentation may restrict the number of government laboratories gathering level and incidence data on perchlorate in the food supply. Concurrently with our MS methodology, we developed an alternative IC method for the more widely available and simpler conductivity detector. Concentration and enrichment of perchlorate were accomplished quickly by passage of cleaned-up food extract through a short anion-exchange high-performance liquid chromatography (HPLC) precolumn, from which column switching injected the retained contents onto an analytical anion-exchange column by coupling the two columns. Use of a graphitized carbon solid-phase extraction (SPE) column efficiently removed potentially interfering conductive coextractives and saved considerable time over published alumina cleanup methods. Perchlorate passed through the column and was collected in a fraction held for instrumental analysis.

Over many years, the FDA has applied column-switching ion-exchange for on-line extraction, enrichment, and partial cleanup in the precolumn to determine cation-forming analytes such as pesticide residues in fruit (*24*, *25*) and the naturally occurring ephedrine alkaloids and synephrine stimulants in dietary supplements (*26*). Unlike UV and fluorescence detectors used in previous work, the suppressed conductivity detector, when optimized to detect a nanogram of perchlorate, severely limited the mobile phase concentration of ionic (hydroxide) eluent that could be neutralized by the self-regenerating suppressor operated at sufficiently low applied current in order to reduce suppressor noise. A reasonable retention time under these conditions could be achieved with columns of considerably lower ion-exchange capacity than in previous work, a disadvantage that would reduce the volume of extract passed through the precolumn before anion breakthrough from heterogeneous anion-exchange equilibrium. Achieving a method determination limit for perchlorate in food that rivaled 1 μg/kg obtained with our IC-MS/MS method was important for data consistency in FDA exposure assessment and risk management programs.

A preconcentration/preelution IC-suppressed CD method for perchlorate in high salinity water (*27*)—later adapted to milk (*13*) and complex samples (*28*)—was similar in instrumental approach. However, we used a less concentrated hydroxide (40 vs 100 mM) mobile phase modified with 5% ACN to shorten chromatographic retention, a self-regenerating suppressor operated at lower current (100 vs 300 mA) for reduced amplitude sinusoidal noise, a lower capacity ion-exchange chromatography column set, and a more efficient extract cleanup provided by graphitized carbon SPE column. Briefly, the edible portion of lettuce, cantaloupe, spinach, or carrot samples was analyzed on a wet weight basis. After sample compositing and chopping, a test portion was blended with dilute acid and filtered, a small volume of measured filtrate was acidified to pH ~2, several milliliters was passed through a carbon SPE column, and perchlorate in an aliquot from a collected fraction was measured. Analysis was complete in hours as compared to days with existing methods based on alumina cleanup. For statistical comparison, an additional analysis was done by IC-MS/MS (*14*) on untreated filtrate diluted with an isotopically labeled perchlorate IS. Also, cooked vegetables processed as baby foods were analyzed for perchlorate.

MATERIALS AND METHODS

Chemicals, Reagents, and Solutions. Nitric acid (70%) and 50% sodium hydroxide solution were analyzed reagents (J. T. Baker, Phillipsburg, NJ). Water resistivity was 18 MΩ cm (Milli-Q cartridge system, Millipore, Billerica, MA). A 1.0 M nitric acid stock solution (90.3 g of reagent per 1 L aqueous solution) was used to prepare a 10 mM nitric acid solution for use as the extraction solvent and diluent for standard solutions. A 100 mM NaOH solution (8.00 g of reagent diluted to make 1 L of aqueous solution), a component of the LC mobile phase, was prepared fresh weekly. Diluted acid and base solutions were stored in wide-mouth polypropylene bottles (Nalgene). A potassium perchlorate stock standard aqueous solution obtained from Alltech (Deerfield, IL) was certified 997 ± 6 μg/mL in ClO_4^-; for clarity, reported standard concentrations were based on the nominal 1000 μg/mL. A 1.00-mL aliquot was diluted to make a 10.0 μg/mL working standard solution, from which perchlorate calibration standards were prepared by serial dilution to make 1.00, 0.100, and 0.0100 μg/mL. Acetonitrile (ACN) and methanol were UV and HPLC grade (Honeywell Burdick & Jackson, Muskegon, MI). Reagent grade Celite 545-AW filtering aid, DOWEX 50WX8-200 cation-exchange resin (H^+ form) and Supelclean ENVI-Carb graphitized carbon SPE columns (500

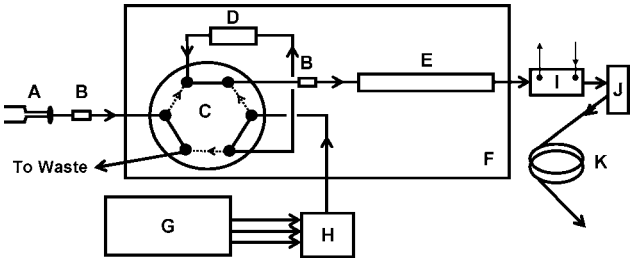

Figure 1. Column-switching ion chromatograph assembled from HPLC syringe and adapter (A), in-line filters (B), injection valve (C) shown in the inject position, precolumn (D), analytical column (E), convective oven (F), solvent degasser (G), multisolvent high-pressure pump (H), anion self-regenerating suppressor (I) in external water mode, conductivity cell (J), and cell backpressure tubing (K). During loading, the test solution followed the dashed-line flow path through the valve and entered the precolumn at the outlet end for the mobile phase to avoid chromatography through the precolumn at injection.

mg per 6 mL) were purchased from Supelco (Bellefonte, PA). The resin was cleaned and conditioned batchwise (50 g) by magnetic stirring with 1.0 M nitric acid for 30 min, followed by ~seven batch rinses with water while stirring for ~15 min each. The final rinse was pH ~5 (indicator strips pH 0−14, EM Science, Gibbstown, NJ). The resin was transferred to 50-mL glass-stoppered flasks, and the water layer was withdrawn by disposable pipet and evaporation until the moist resin would clump onto a small spatula for removal. Carbon SPE columns were conditioned as needed with one column volume each of methanol and extraction solvent.

Instrumentation. The column-switching ion chromatograph shown in **Figure 1** was assembled from a gradient pump and vacuum membrane degasser (respectively, models P4000 and SCM 1000, ThermoFinnigan, San Jose, CA), an electrically actuated injection valve (model E4C6W, VICI, Houston, TX) mounted inside a convective column oven (old DuPont Instruments or equivalent), an anion self-regenerating suppressor (ASRS, ULTRA II, Dionex, Sunnyvale, CA) operated in the external water mode, and a Dionex thermostated conductivity cell (model DS3 Detection Stabilizer) with electrochemical detector (model ED40). A coil of PEEK tubing (0.010 in. i.d. × 2−2.5 ft) provided backpressure (30−40 psig) to suppress air bubble formation in the cell. Filters (A-316, Upchurch Scientific, Oak Harbor, WA) with replaceable 0.5-μm frits (A-103x) were installed before the valve's inlet filling port and before the analytical column.

A Dionex IonPac AG16 anion-exchange guard column (4 mm × 50 mm) functioned as a precolumn to extract and enrich perchlorate and other anions from test solutions for injection (reverse flush) onto an AS16 anion-exchange analytical column (4 mm × 250 mm). The columns, chosen for their greater ion-exchange capacity than the comparable 2 mm i.d. set, were held at 40 °C. During injection, the coupled columns were eluted at 1.0 mL/min with a mobile phase dynamically generated from (A) 100 mM NaOH, (B) water, and (C) ACN at 40:55:05 (A−B−C). During loading of the precolumn, 0.5 mL of water was pushed through to flush out the mobile phase and then 50−750 μL of test solution was delivered, followed by another 0.5 mL of water. The ASRS was operated at 100 mA, and a 4−5 mL/min water regenerant flow rate was measured with the current on. Conductance of the suppressed background signal inside a 40 °C cell was 2.2−3.2 μS with the suppressor noise averaging 0.5−0.8 nS peak-to-valley. The detector signal was acquired and processed by a chromatography data station (model SS420X analog-to-digital converter, Scientific Software, Inc., Pleasanton, CA; Chromquest ver. 4.0 software, ThermoFinnigan).

Analysis. Most samples of lettuce (iceberg, green leaf, red leaf, and romaine varieties), cantaloupe, spinach, and carrots were collected by FDA inspectors from growers or packing sheds as part of the 2004 "Collection and Analysis of Food for Perchlorate", a 500 sample field assignment. Samples (5−20 lb each) were shipped overnight to FDA's Southeast Regional Laboratory, for compositing in a food chopper and analysis by IC-MS/MS methodology (*14*, *29*). At our request, frozen (−20 °C) portions of certain sample composites were sent to us for IC-CD method development research and quality assurance check analysis. Additional samples (1−2 lb each), especially winter lettuce from the 2004 crop, were purchased locally at suburban Washington, DC, supermarkets, chopped in a food processor, and stored at −20 °C. Samples were thawed before a test portion was removed for analysis.

Glassware was cleaned in perchlorate-free detergent (MICRO-90, International Products Corp., Burlington, NJ) and rinsed with extraction solvent before use. A 100-g test portion (weighed to 0.01 g) of thawed, thoroughly mixed sample and ~10 g of Celite (omitted for iceberg lettuce samples) were blended with 150 mL of 10 mM HNO_3 in a Waring glass jar (rubber gasket replaced with PTFE) for 6 min at high speed. The contents plus two 20-mL rinses of the jar were filtered under vacuum through a rinsed glass fiber filter (#30, 11 cm, Schleicher & Schuell MicroScience, Inc., Riviera Beach, FL). Volume recovery was usually complete in 30 min, but filtration of some extracts exceeded 90 min, despite use of the filtering aid. The filtrate volume was measured. A portion (25 mL) was poured into a beaker and stirred magnetically while cation-exchange resin was added until pH ~2 was reached (combination glass electrode, model 91−55, Thermo Orion, Beverly, MA), after which stirring continued for 15 min. After settling, supernatant was poured onto a conditioned ENVI-Carb SPE column placed on a vacuum manifold (Visiprep, Supleco), two 2-mL fractions were collected, and an aliquot of the second fraction was analyzed by IC-CD. The maximum aliquot size depended upon the crop: 400 μL for spinach; 500 μL for red leaf lettuce; and 750 μL for cantaloupe, carrots, and lettuce of iceberg, green leaf, and romaine varieties. Raw and treated extracts kept well for days stored in a refrigerator.

The IC columns, stored in water, were cleaned of strongly retained crop material before daily use by pumping 100 mM NaOH (base) at 1.5 mL/min for 30 min, followed by a 5-min gradient to 35:65 base/water (hold 15 min) and 1.0 mL/min flow rate. The ASRS and conductivity cell were disconnected from the system. Note that when cooked foods had been analyzed, columns were cleaned with 10:90 base/ACN mobile phase for 30−60 min at 1.0 mL/min. Daily calibration began after equilibrating the columns to the 1.0 mL/min 40:55:05 mobile phase proportioned from base, water, and ACN, respectively. The perchlorate retention time was stable during the day. Note that mobile phases were never premixed and 100 mM NaOH was prepared fresh at least weekly.

A six-point linear regression calibration of peak area was performed at 50.0, 30.0, 10.0, 5.00, 2.50, and 1.00 ng of perchlorate in this order using 50−500-μL aliquots of calibration standard solutions. The low-frequency sinusoidal suppressor noise pattern modulating the detector signal often caused automatic integration to begin and end the perchlorate peak at a valley point of the noise cycle, a positive integration bias that included the full peak-to-valley noise in the baseline. If needed, peak start and end points were reset manually at the noise cycle midpoint. The mass of perchlorate Q (ng) in the analyzed test solution TS (mL) was quantitated from peak area A and the linear regression coefficients slope m and intercept b according to $Q = (A - b)/m$. Determination (μg/kg) of perchlorate in the sample was calculated from $(Q/TS)(V/W)$, where V was the final extract volume (mL) and W was the test portion weight (g).

Comparative Analysis by IC-MS/MS. Slight changes were made to the method (*14*) to compensate for perchlorate losses experienced with some disposable syringe filters. Filtrate with no cleanup was diluted 9:10 with 1.00 mL of 0.100 μg/mL of aqueous $Cl^{18}O_4^-$ IS in a 15-mL graduated (0.1 mL) glass-stoppered centrifuge tube. Diluted solution was drawn up into a syringe and pushed through a 0.2-μm nylon filter (30 mm, Titan2, Sun-SRi, Duluth, GA), diverting ~six drops to waste before collecting 0.5−1.0 mL in an autosampler vial.

RESULTS AND DISCUSSION

Chromatography. Suppressed conductivity chromatograms in **Figure 2** compare extracts and the 1.0 ng lowest calibration standard, which at S/N ~ 10 was the measurement limit of quantitation (LOQ). Perchlorate eluted ~11 min, separated from similar-sized peaks and far away from intense peaks, in a chromatographic region supportive of accurate integration.

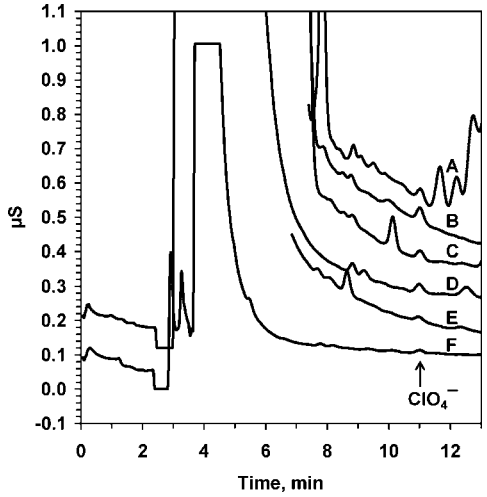

Figure 2. Suppressed conductivity IC of extracts prepared from the edible portion of (A) cantaloupe with seeds, (B) cantaloupe without seeds, (C) carrots, (D) iceberg lettuce, and (E) spinach. Samples had 7–14 μg/kg incurred perchlorate. Chromatographic analysis was done on 750 μL except spinach, which was 400 μL. For comparison, a chromatogram of (F) 1.0 ng of perchlorate standard is included.

Quantitation of the suspected perchlorate peak in these food extracts exceeded the measurement LOQ and was equivalent to a sample level in the 7−14 μg/kg range. Findings were confirmed by IC-MS/MS with the following comparative results (μg/kg CD vs MS/MS): (a) cantaloupe with seeds, 9.64 and 9.87; (b) seedless cantaloupe, 14.0 and 13.9; (c) carrots, 7.39 and 9.22; (d) iceberg lettuce, 10.5 and 8.69; and (e) spinach, 9.59 and 12.6. Determinative results were within ±25% relative to mass spectrometry, with no evidence of systematic high bias due to conductive coeluting crop material. Chromatography of cantaloupe extract was very long, mainly due to coextractives from seeds included in the composited sample; analysis of just the fleshy fruit produced a cleaner, simpler chromatogram (cf. traces A and B).

Graphitized carbon removed organic material while allowing perchlorate and ions to pass through the column for collection and analysis. Organoanions, conceivably conjugate bases of organic acids, potentially interfered with conductivity measurement. Therefore, to suppress weak acid ionization and thereby enhance likelihood of removal by carbon adsorption of the neutral form, the pH was lowered to ~2 before cleanup. Extract acidification by release of resin-bound hydrogen ions exchanged for metal ions introduced no additional anion to compete with perchlorate for the fixed number of anion-exchange sites on the precolumn stationary phase. Alternatively, the addition of an acid solution, assuming minimal dilution, would increase the anion concentration and thus reduce the aliquot size for precolumn extraction and enrichment under nonequilibrium conditions; moreover, a smaller quantity of perchlorate injected onto the analytical column would raise the LOQ. Once the precolumn reached heterogeneous ion-exchange equilibrium, proportionality between analyte signal and volume passed was lost, quantitation became independent of aliquot size, and determination failed.

The average method LOQ for perchlorate determination in a crop was calculated from the 0.0010 μg measurement LOQ assumed to be in the recommended extract volume passed through the precolumn, multiplied by the average final volume for that crop (values ranged from 247 to 270 mL), and divided by the test portion weight (0.100 kg). For example, the method LOQ for cantaloupe averaged 3.5 μg/kg [i.e., (0.0010 μg/0.750 mL) × (266 mL/0.100 kg)]. Method LOQs (μg/kg) for the other crops were 3.3 for carrots; 3.6 for iceberg, green leaf, and romaine lettuce but 5.0 for red leaf lettuce; and 6.4 for spinach.

Table 1. Recovery (%) of Perchlorate Added at 10 μg/kg to Crop Controls[a]

crop	trial 1	trial 2	average
cantaloupe	106	80.5	93.3
carrots	95.6	95.9	95.8
lettuce	97.6	95.1	96.4
spinach	85.0	77.5	81.3

[a] % recovery = $[(F - U)/A] \times 100$, where F and U are determinations (μg/kg) of perchlorate in the fortified and unfortified test portions, respectively, and A is the concentration added. U = 3.61 for cantaloupe; 3.17 and 3.41 for carrot trials 1 and 2, respectively; 0 (not detected) for lettuce; and 7.64 for spinach.

Calibration and Recovery. Calibration of peak area response (y) from suppressed CD of 1−50 ng of perchlorate (x) was described by the following linear regression equation (mean ± SD) for $N = 51$: $y = (1.55 \pm 0.088) \times 10^5 x - (0.32 \pm 0.20) \times 10^5$. Over this long period, the RSD of the slope was ±6%, and the 95% confidence limits (mean ± $t_{df=50}$·SD) about the intercept included zero. Further evidence supporting consistently linear calibration came from goodness-of-fit R^2 averaging 0.9997 ± 0.0003. Results of duplicate recovery trials at the 10 μg/kg fortification level are presented in **Table 1** after correction for incurred level (only control lettuce was blank). The corrected recovery averaged 93−96% for cantaloupe, carrots, and lettuce and 81% for spinach. The spinach recovery values may have been influenced by a high control level correction obtained by single analysis, which insufficient sample prevented checking. The grand mean (corrected) recovery of 91.7% and RSD of 10.6% ($N = 8$) were acceptable at 10 μg/kg.

Comparative Analysis. Determinative accuracy was tested statistically against the result by IC-MS/MS analysis of raw filtrate (no cleanup) with $^{18}O_4$-labeled perchlorate added as an IS. Linear regression plots of determination by IC-CD against the gold standard IC-MS/MS are shown in **Figure 3** for 17 cantaloupe samples, 10 carrot samples, 27 lettuce samples (four

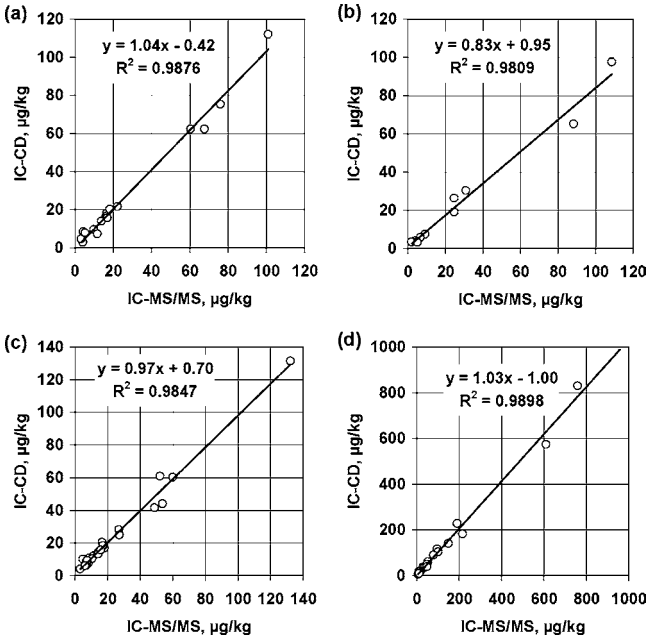

Figure 3. Linear regression statistics for perchlorate determination by conductometric vs mass spectrometric measurements for (**a**) cantaloupe, (**b**) carrots, (**c**) lettuce (four varieties), and (**d**) spinach sample sets.

Table 2. Perchlorate Findings between 3 and 11 μg/kg by IC-CD As Compared to Independent Analysis by IC-MS/MS

crop	IC-CD (*y*)	IC-MS/MS (*x*)	difference (*y* – *x*)
cantaloupe	2.78	4.19	–1.41
carrots	3.17	4.98	–1.81
carrots	3.41	1.89	1.52
lettuce	4.00	3.33	0.67
carrots	4.05	3.63	0.42
cantaloupe	4.58	3.21	1.37
spinach	5.13	7.33	–2.20
carrots	5.71	6.59	–0.88
spinach	5.77	8.43	–2.66
lettuce	6.04	6.31	–0.27
lettuce	6.27	7.06	–0.79
cantaloupe	7.26	11.8	–4.54
lettuce	7.38	8.31	–0.93
carrots	7.39	9.22	–1.83
spinach	7.64	8.32	–0.68
cantaloupe	7.80	5.28	2.52
lettuce	8.18	6.93	1.25
cantaloupe	8.49	4.30	4.19
lettuce	8.54	7.61	0.93
lettuce	9.25	7.51	1.74
spinach	9.59	12.6	–3.01
cantaloupe	9.64	9.87	–0.23
lettuce	10.1	4.97	5.13
lettuce	10.2	10.5	–0.30
lettuce	10.5	8.69	1.81
lettuce	10.6	9.02	1.58
		N	26
		mean	0.060
		SD	2.185
		t	0.143
		P value	0.888

varieties included), and 22 spinach samples. Goodness-of-fit R^2 was >0.980 in all crops, indicating that results by IC-CD were a strong predictor of the results by IC-MS/MS. Bias, however, was indicated by regression coefficients that differed from unity slope and zero intercept. Proportional (±4%) and fixed (±1.00 μg/kg) bias were small for cantaloupe, lettuce, and spinach. However, a large negative proportional bias (17%) was observed for carrots, although the fixed bias was similar to the other crops. One data point (88.4, 65.2) was responsible for 7% of this bias (i.e., $m = 0.90$ if these data were omitted), but no justification for dropping the data was evident. Doubling the number of carrot samples to bring the set size in line with the other crops may provide better regression statistics later. Our data indicate that about one-third of the spinach samples exceeded 100 μg/kg as compared to only one sample of cantaloupe, carrot, or lettuce. The highest perchlorate finding in this study was ~800 μg/kg in spinach.

A two-tailed paired Student's *t*-test was performed to test the null hypothesis that there was no difference between the means of the IC-CD and IC-MS/MS determinations at the 95% confidence level (*P* value > 0.05). Carrots showed no statistically significant difference between the means of CD and MS/MS determinative values ($P = 0.12$), despite large deviation from the ideal regression slope. Likewise, the null hypothesis was true for cantaloupe ($P = 0.51$), spinach ($P = 0.55$), and lettuce ($P = 0.93$). Therefore, when a sample set of these crops is analyzed by the IC-CD method, the sample set average determination 95% of the time will be no different from that average obtained had the IC-MS/MS method been used, whenever perchlorate exceeded the IC-CD method LOQ.

Presented in **Table 2** are comparative findings of low-level (<11 μg/kg) perchlorate in crops culled from the results displayed in **Figure 3**. The mean difference (0.06 μg/kg) between determinative results was not significant at the 95% confidence level (*P* value = 0.89). Statistically, determination by IC-CD was equivalent on average to determination by IC-MS/MS. Nevertheless, on an individual analysis basis, comparative results differed up to ±5 μg/kg, and triple quadrupole mass spectrometry with labeled perchlorate IS provided the best specificity and accuracy. If the average of perchlorate findings in a sample dataset is sought, then the IC-CD method may be fit-for-purpose. Although the conductivity detector was not specific for perchlorate, the carbon column cleanup and column-switching IC appeared to impart high selectivity to perchlorate detection by removing endogeneous conductive interferences.

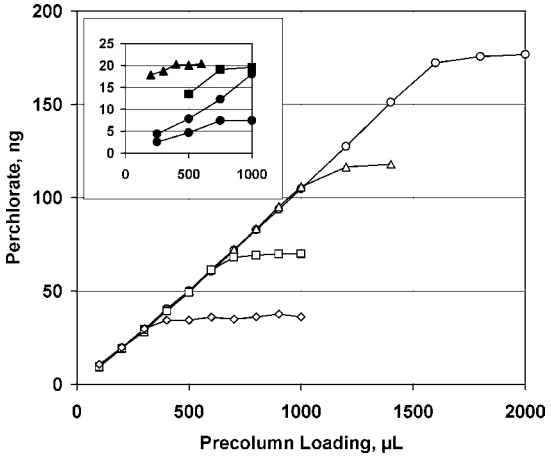

Figure 4. Perchlorate uptake by the precolumn from test solutions of various anion concentrations. Main graph: plots for 100 ng/mL perchlorate standard solutions prepared in nitric acid concentrations of (○) 10, (△) 15, (□) 25, or (◇) 50 mM. (Inset) Plots for extracts prepared from (●) green leaf lettuces, (■) iceberg lettuce (offset 2 μg/kg), and (▲) spinach (offset 15 μg/kg).

Ruggedness. Extending the method to other crops must be done cautiously. The conductivity detector provided no molecular fingerprint for identification and always will be susceptible to false positives or interference from conductive crop material eluting with perchlorate. This method was also susceptible to negative bias from samples that provided large quantities of coextractive anions that competed with perchlorate for the 3.5 μequiv of AG16 exchange sites. For these crops, the precolumn and extract ion-exchange (IX) equilibrated sooner. This condition is explained by a simple case of univalent−univalent anion exchange between P^- (predominant level) and T^- (trace level) in solution and a solid phase ion exchanger (IXer) initially in the P-ion form. At heterogeneous IX equilibrium, the IXer ceased uptake of T from solution, its concentration in the solid phase fixed according to $[\bar{T}] = K_{IX}[\bar{P}][T]/[P]$, where the overhead bar refers to the IXer phase and K_{IX} is the IX equilibrium constant. Because T was a trace, then $[\bar{T}] + [\bar{P}] \approx [\bar{P}] = \bar{C}$, the IX capacity, and $[\bar{T}] = K[T]/[P]$, where constant $K = K_{IX}\bar{C}$. Therefore, for a $NO_3^- - ClO_4^-$ (P−T) system, the perchlorate enrichment factor $[\overline{ClO_4^-}]/[ClO_4^-]$ at IX equilibrium was inversely proportional to $[NO_3^-]$, a constant. Before equilibrium, perchlorate uptake by the exchanger proceeded linearly with depletion in solution passed through the precolumn because of fast ion-exchange kinetics and lack of evidence of a competing separation mechanism (e.g., perchlorate does not form complexes).

Ion-exchange dynamics in the precolumn are plotted in **Figure 4** at various loadings of a perchlorate standard solution (~100 ng/mL) prepared in 10, 15, 25, or 50 mM nitric acid. As predicted, perchlorate enrichment increased linearly regardless of nitrate level until IX equilibrium, which was reached at

Table 3. Perchlorate Findings in Vegetable Baby Foods

baby food[a]	perchlorate (μg/kg) IC-CD	IC-MS/MS
carrots #1	1.6 < LOQ	2.93
carrots #2[b]	19.8	21.2
carrots #3	3.60	1.91
carrots #4	7.76	8.32
carrots #5[b]	2.7 < LOQ	2.92
carrots #6	ND	2.59
carrots #7	1.4 < LOQ	ND
carrots and sweet peas	3.16	2.31
garden vegetables	13.0	17.1
mixed vegetables	ND	1.44
creamed spinach[c]	47.0	56.2

[a] Carrot nos. 1–3 were life stage 1; all other samples were life stage 2. [b] Organically grown. [c] Test portion reduced to 50 g to improve vacuum filtration.

smaller loadings as nitrate levels increased. At 10 mM, the concentration chosen for extraction solvent, up to ~1600 μL of standard solution could be loaded before equilibration. Although choice of a higher concentration may have simplified the procedure if additional acidification before SPE cleanup were unnecessary, the severe reduction in loading (e.g., ~300 μL at 50 mM nitric acid) would have elevated the LOQ proportionately. Therefore, adding cation-exchange resin to crop extract to bring the pH of 4−6 down to ~2, although tedious, permitted perchlorate determination in field crop samples rivaling that of mass spectrometry. Maximum loadings of extract were a fraction of that for perchlorate standard in extraction solvent because the crop contributed exchange-competitive anions. The inset plots in **Figure 4** show two samples of green leaf lettuce with different maximum loadings easily explained by differences in concentration of exchange-competitive anions. To build in a safety margin, the recommended maximum loading for green leaf lettuce extract was 750 μL, also true for romaine and iceberg varieties. Spinach, comparatively high in mineral content (*30*), was expected to boost anion levels in the extract; therefore, a reduction in maximum loading to 400 μL was not surprising. When applying the method to a new commodity, samples were analyzed from different growing regions, and various loading volumes were tested before deciding on an acceptable maximum. Perchlorate determination was independent of volume passed through the precolumn equal to or below a safe maximum loading.

The method was tested with cooked vegetables containing carrots or spinach processed as baby foods. Jars of each item were purchased locally in units of the same lot to provide ~150 g of combined sample. Processed vegetables behaved differently through the method. For example, the test portion size of creamed spinach was halved to 50 g so that filtration of the extract was possible. Cleaned-up extract of baby food carrots was yellow instead of water clear as observed for raw carrots (and raw agricultural commodities in general). Late chromatographic eluters lengthened the analysis of processed food. However, colored pigments and other strongly retained crop materials were readily cleaned off the columns with 10:90 100 mM NaOH/ACN (dynamically proportioned) mobile phase. Perchlorate findings in 11 products are displayed in **Table 3** along with determination by IC-MS/MS (1 μg/kg method LOQ). The determinative means of the six samples above the IC-CD method LOQ of 3 μg/kg were not significantly different at 95% confidence (two-tailed paired *t*-test; P value = 0.25). About two-thirds of the samples had <4 μg/kg of perchlorate, but only one had nondetectable perchlorate by MS/MS. Perchlorate in one sample of organically grown carrots was ~20 μg/kg but in another sample was below the IC-CD method LOQ. The highest perchlorate concentration (~50 μg/kg) was found in creamed spinach, and the third highest (~15 μg/kg) was found in garden vegetables, a blend of spinach with carrots and peas, which were consistent with earlier analyses that found perchlorate in raw agricultural spinach samples typically at much higher levels than in carrots, lettuce, and cantaloupe samples.

In summary, perchlorate was determined in selected crops and vegetable baby foods by a simple, inexpensive, suppressed conductivity method, involving a quick carbon column cleanup of acidified extract, precolumn on-line enrichment, and subsequent injection by column-switching IC. Statistically, determination on average was no different from IC-MS/MS by fixed volume injection of untreated extract, although the IC-CD method was slower because of cleanup steps. Single-digit method LOQs rivaled the 1 μg/kg achieved by MS/MS. External standard calibration was reliable and sufficient for conductometric quantitation. In contrast, the perchlorate signal from MS/MS with ESI was affected by the matrix and required compensation by an isotopically labeled perchlorate added as an IS.

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