METHOD 6850

DETERMINATION OF PERCHLORATE USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY/MASS SPECTROMETRY (LC/MS)

USEPA Region 6 QA Conference
Dallas, TX
October 20, 2004

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USEPA OSW
Inorganics Method Development

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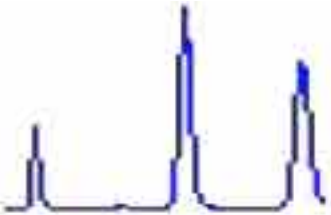

ANALYSIS OF PERCHLORATE

Perchlorate Testing Roundtable
USEPA Region 6 QA Conference
Dallas, TX
October 23,2003

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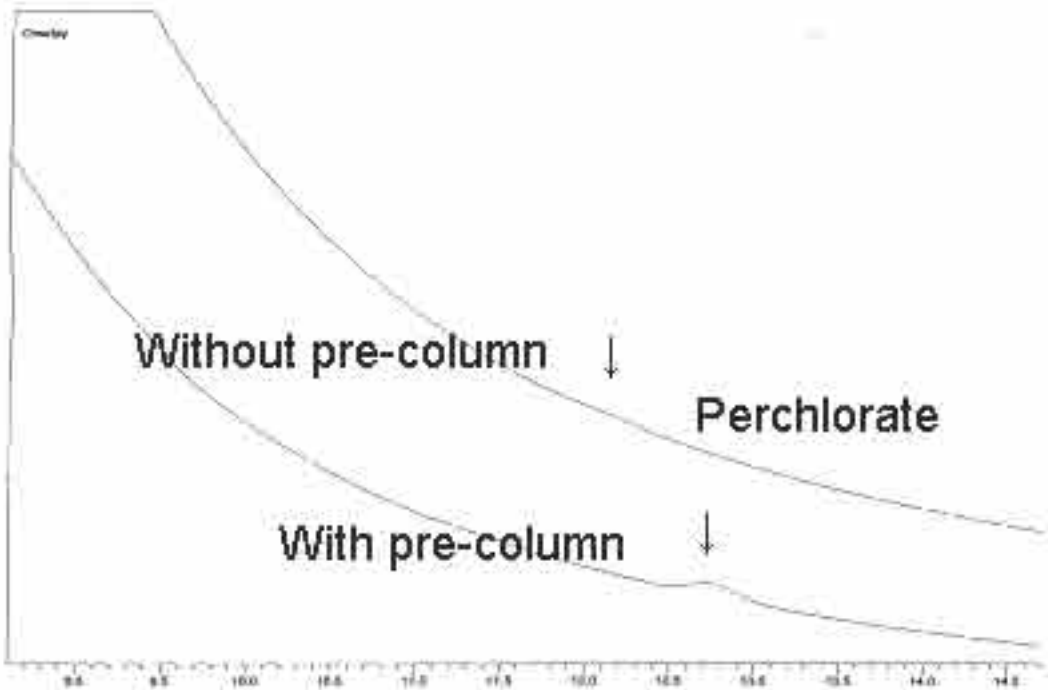
Chromatograms

Overlay of 21,500 uS/cm matrix with and without pre-column at 20 ug/L



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LC/MS Method Development

Liquid Chromatography is preferable to Ion Chromatography for direct injection methods without using a precolumn to remove matrix interferences.

LC/MS method will selectively retain Perchlorate for analysis.

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LC/MS Analysis of ClO4

By Kham Lin, K'(Prime) Technologies
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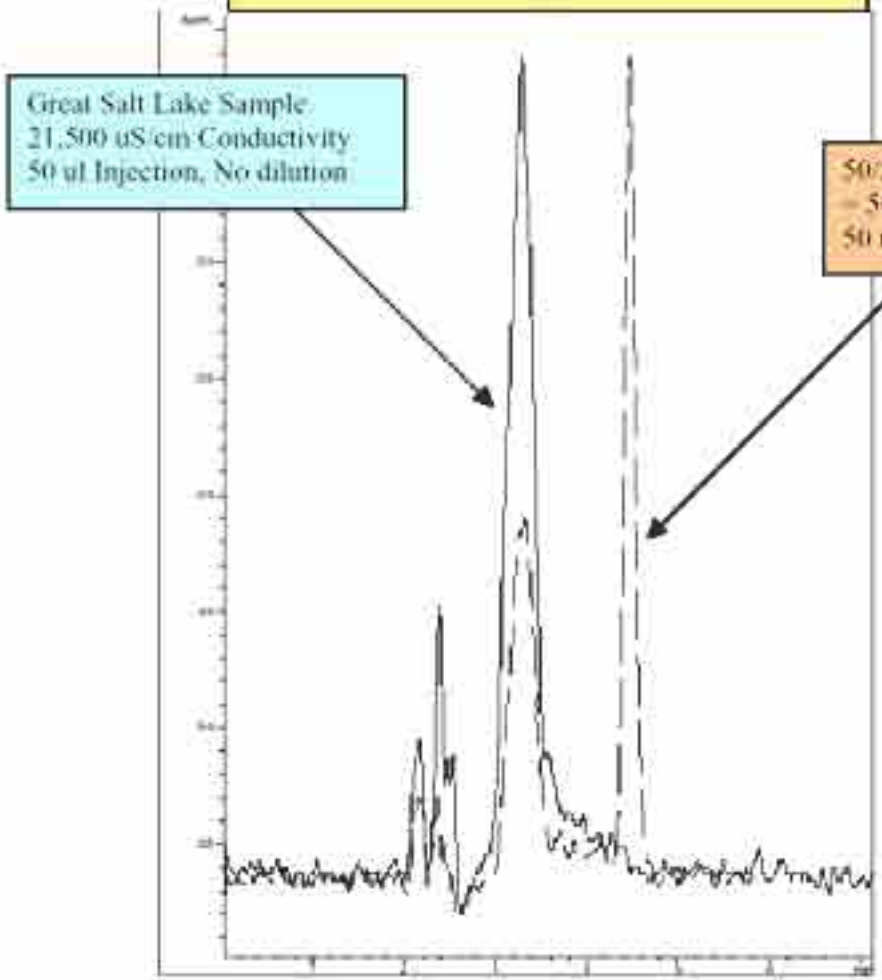

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Agenda

- Instrumentation and Sample Preparation
- Perchlorate Identification
- Chromatograms
- Detection and Quantitation Levels
- Method Validation
- 6850 Quality Control
- 6850 Future Plans

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Instrumentation and Sample Preparation

- Agilent 1100 LC/MSD
- Mobile phase: Acetonitrile, Water, Acetic Acid
- Column: K'(Prime) Technologies, Inc, KP-RPPX series

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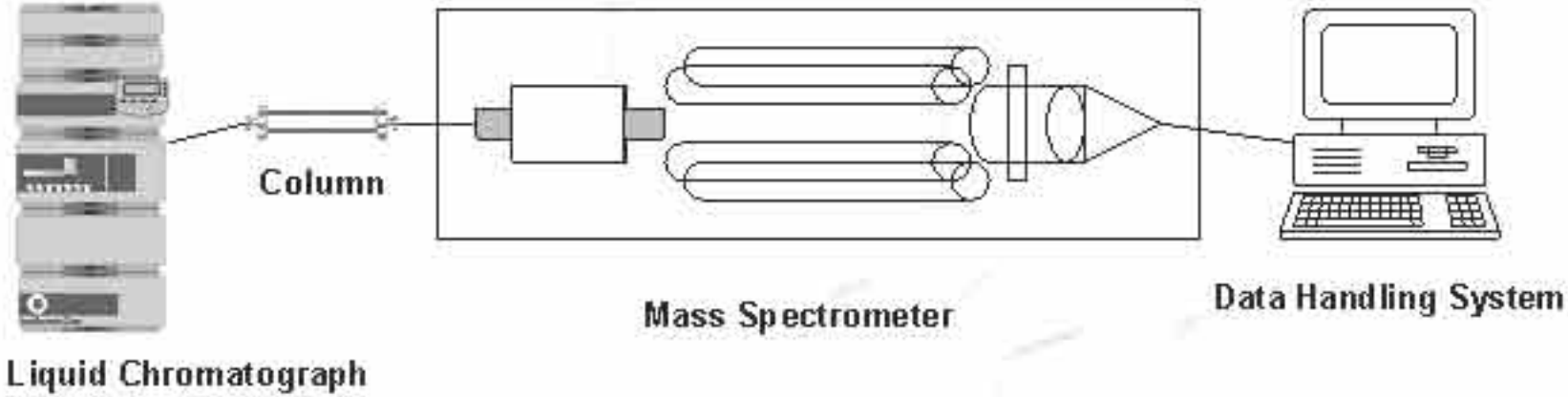

Components:

Binary Pump G1312A
Micro-degasse G1279A
Autosampler G1313A

Column Compartment G1316A
Agilent 1100 LC/MSD G2708DA
Agilent LC/MSD Chemstation G2710AA

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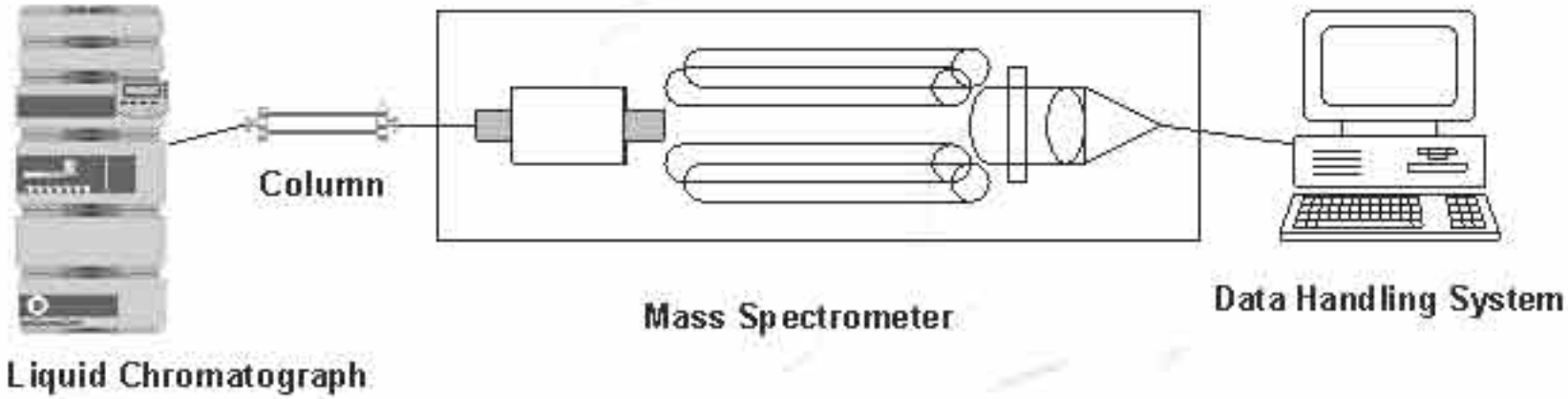

Pump Control

Flow Rate: 0.5 mL/min
Run Time: 13.0 min
HPLC Mobile Phase:
Isocratic
53.00%, Solvent A (95% ACN / 4.5% Water / 0.5% Acetic Acid)
47.00%, Solvent B (94.5% Water / 5% ACN / 0.5% Acetic Acid)

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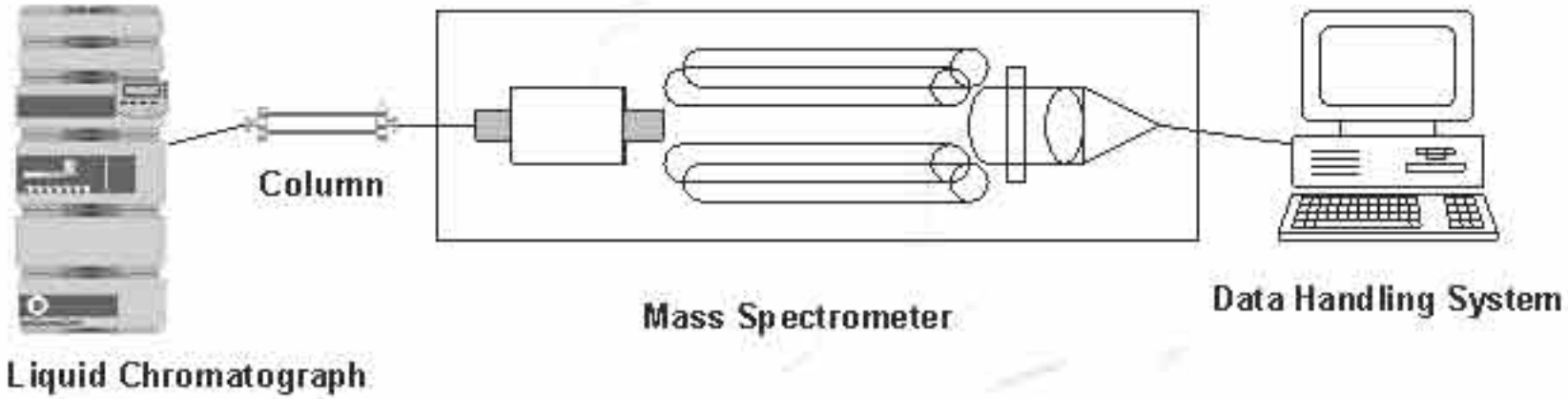

Mass Spectrometer:

Ionization Mode: Electrospray
Polarity: Negative
SIM Parameters:

SIM Ion	Fragmentor	Gain(EMV)	Actual Dwell
83.00	160V	3.0	192 msec
85.00			192 msec
89.00			192 msec

Spray Chamber:

Gas Temp:	320°C
Drying Gas (Nitrogen):	12.0 L/min
Nebulizer Pressure:	50 psig
Capillary Voltage:	Negative: 1450 V

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Instrumentation and Sample Preparation

Matrix	Sample Description
Drinking Water (DW)	Laboratory Distilled Water Conductivity = 1 µS
Soil	Soil
Biota	Grass
Synthetic Ground Water (SGW)	Laboratory Distilled Water with 1000 mg/L of chloride, sulfate, and carbonate. Conductivity = 7700 µS
Great Salt Lake (GSL)	Water taken from the Great Salt Lake and diluted ten fold. Conductivity = 21000 µS

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Instrumentation and Sample Preparation

Water samples are prepared by adding an aliquot of sample to a 15-mL disposable centrifuge tube. An appropriate aliquot of internal standard and glacial acetic acid is added to each sample. Each sample is filtered through a 0.45-µm filter into an autosampler vial for analysis.

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Instrumentation and Sample Preparation

Soil samples are prepared by adding an aliquot of sample and 10 mL of ASTM Type II water to a 15-mL centrifuge tube. An appropriate aliquot of internal standards and glacial acetic acid is added to each sample. The mixture is vortexed, sonicated for at least 10 minutes, and vortexed again. If necessary, the sample is centrifuged. The extract is then filtered through a 0.45-µm filter into an autosampler vial for analysis.

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Instrumentation and Sample Preparation

Biota (Plant) samples are prepared by using a sufficient portion (at least 10 grams) of sample and ground through a hand-operated stainless steel grinder. ASTM Type II water is added to an aliquot of biota sample in a 50-mL centrifuge tube. An appropriate aliquot of internal standard and glacial acetic acid are added to each sample. The mixture is vortexed and left overnight, which allows for complete saturation of the sample. Prior to analysis, the sample is vortexed again, then centrifuged at 5000 rpm for 30 minutes. A portion of the supernatant is then drawn through an activated C18 column, which removes a large portion of organic contaminants. The final extract is then filtered through a 0.45-µm filter into an autosampler vial for analysis.

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Perchlorate Identification

- ✓ Perchlorate at mass 83
- ✓ Perchlorate 83/85 Isotopic Ratio
- ✓ ^{18}Oxygen Labeled Perchlorate as Internal Standard

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Perchlorate Identification

✓ Perchlorate at mass 83

Mass spectrometry is used to monitor Perchlorate at mass 83, which is achieved by the partial fragmentation of Perchlorate to remove an oxygen atom. Using mass 83 eliminates known interference caused by sulfate at mass 99.

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Perchlorate Identification

✓ Perchlorate 83/85 Isotopic Ratio

Confirmation of Perchlorate is obtained using the naturally occurring isotopic ratio of ^{35}Cl to ^{37}Cl, which is 3.065, to monitor the ratio of mass 83 and 85 from Perchlorate.

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Perchlorate Identification

✓ ^{18}Oxygen Labeled Perchlorate as Internal Standard

Isotopic ^{18}Oxygen labeled Perchlorate is used as an internal standard and added to each standard and sample. This internal standard is used for relative retention time confirmation, monitoring instrument performance, and internal standard calibration.

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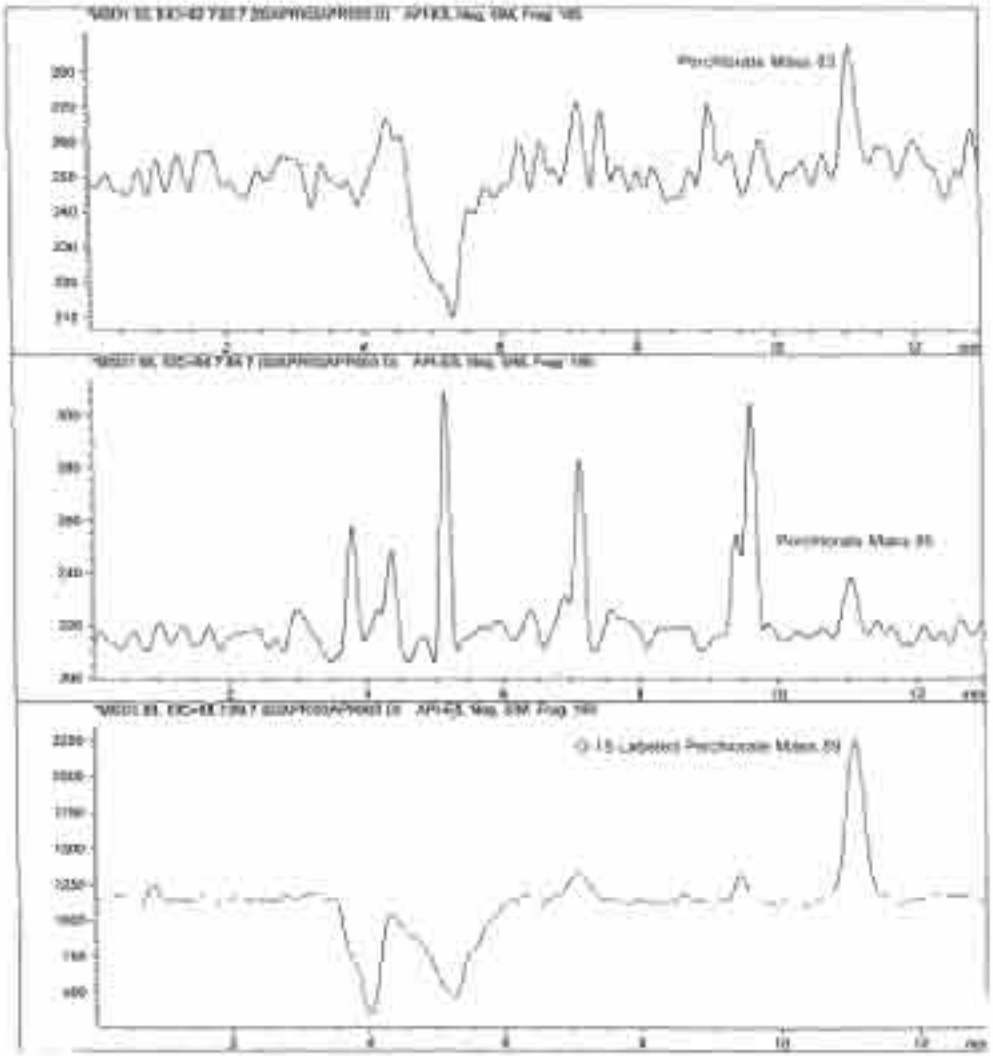
Chromatogram
Drinking Water
Matrix

0.2 ug/L



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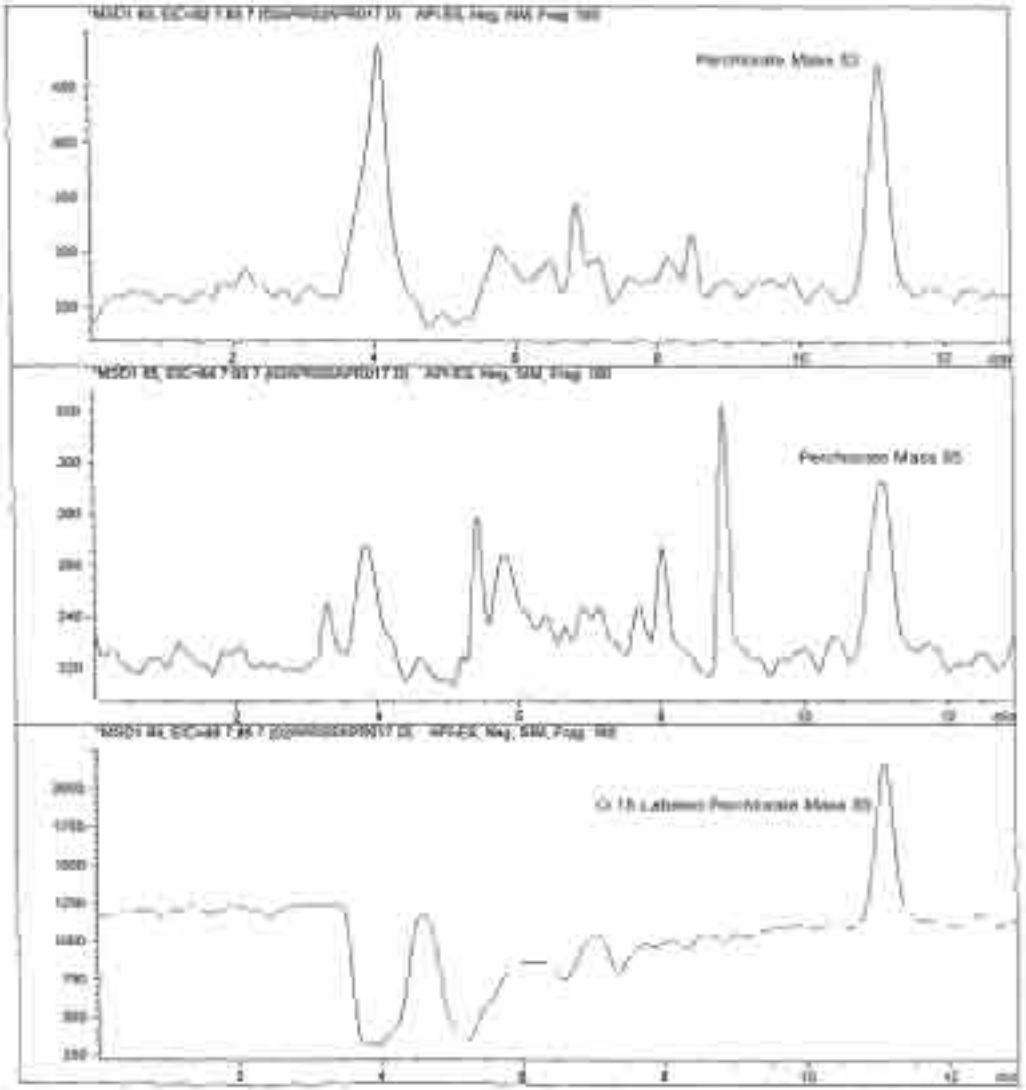
Chromatogram
Soil Matrix

10 ug/Kg



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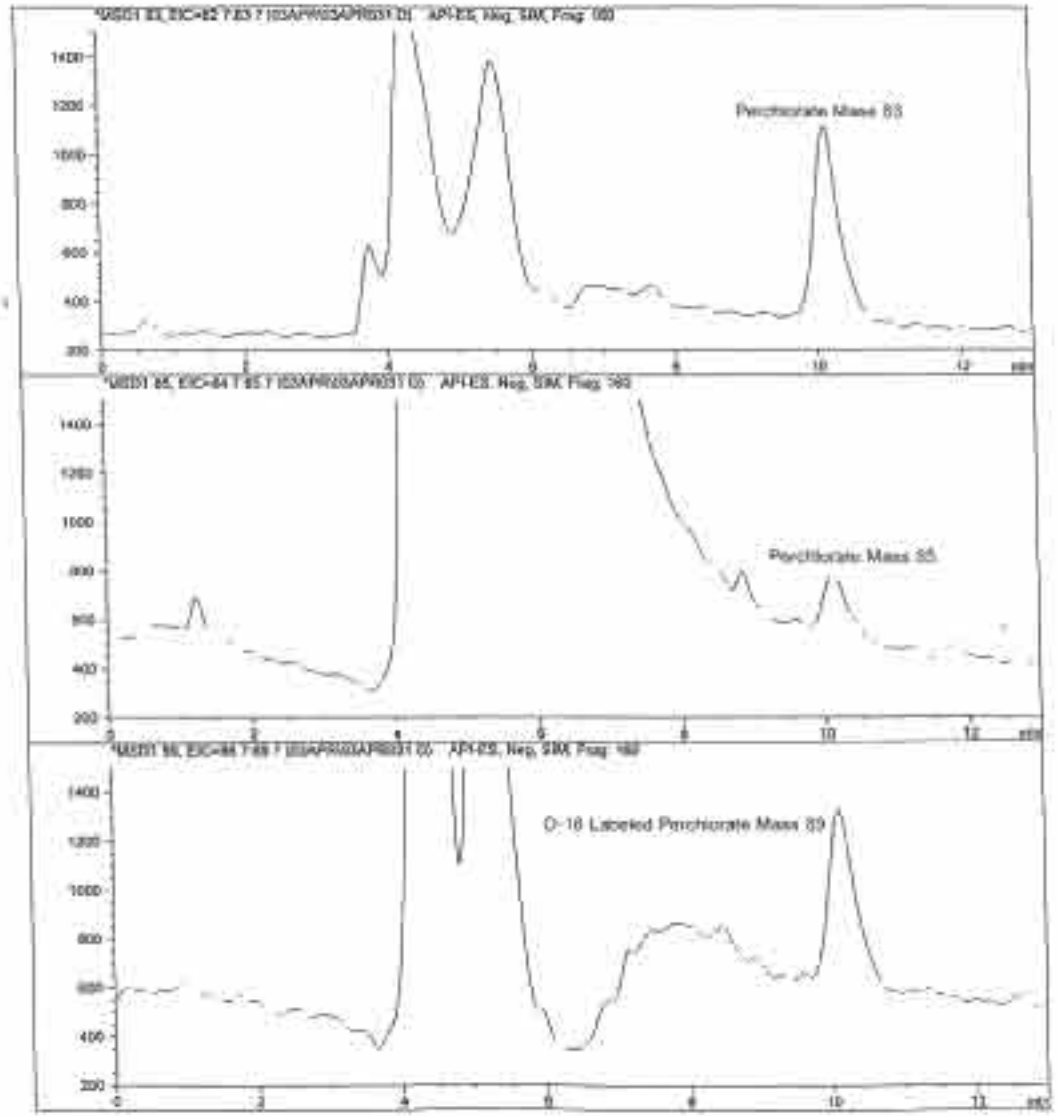
Chromatogram
Biota Matrix
(Grass)

50 ug/Kg



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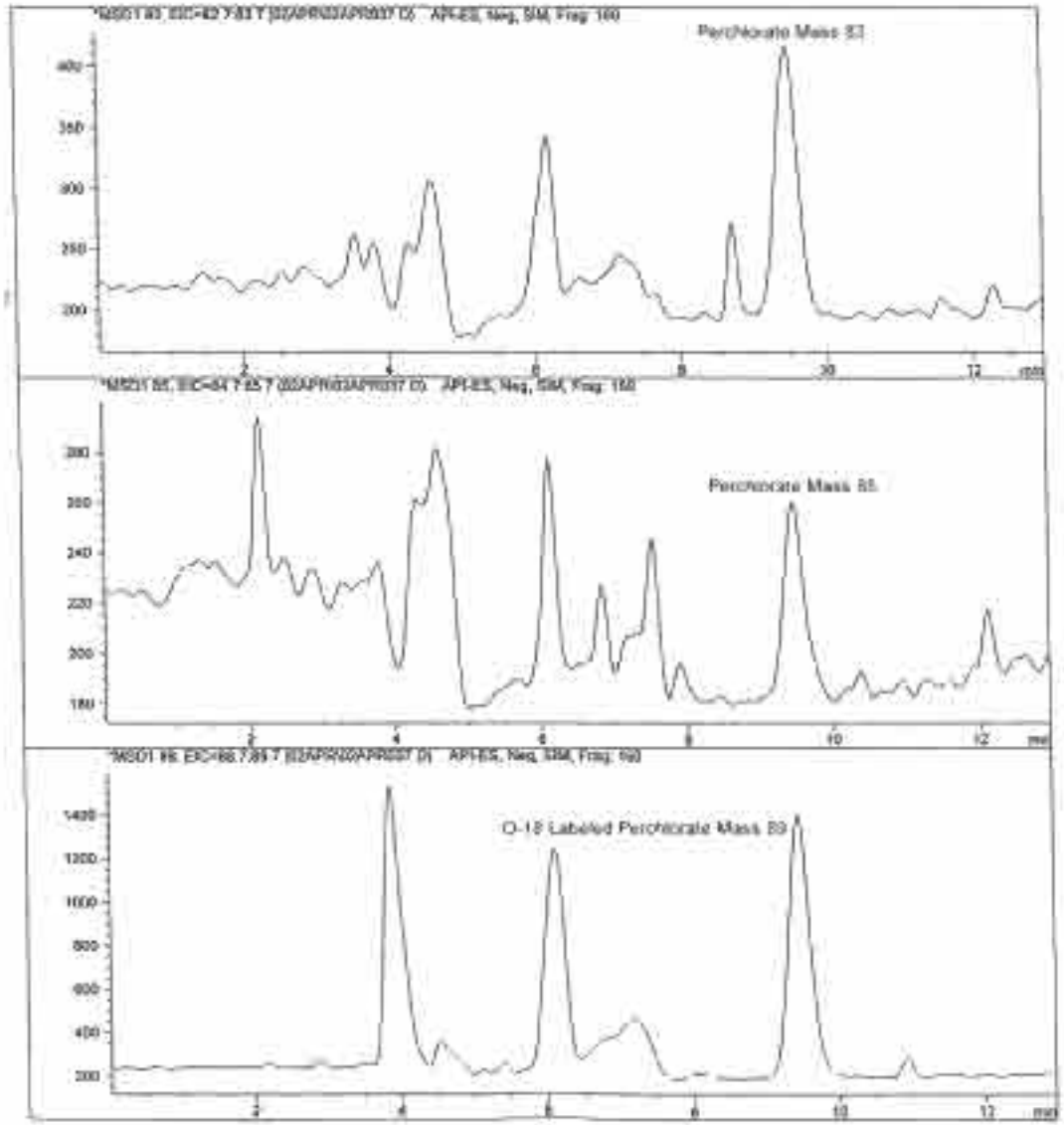
Chromatogram
Simulated
Ground Water
Matrix

1.0 ug/L



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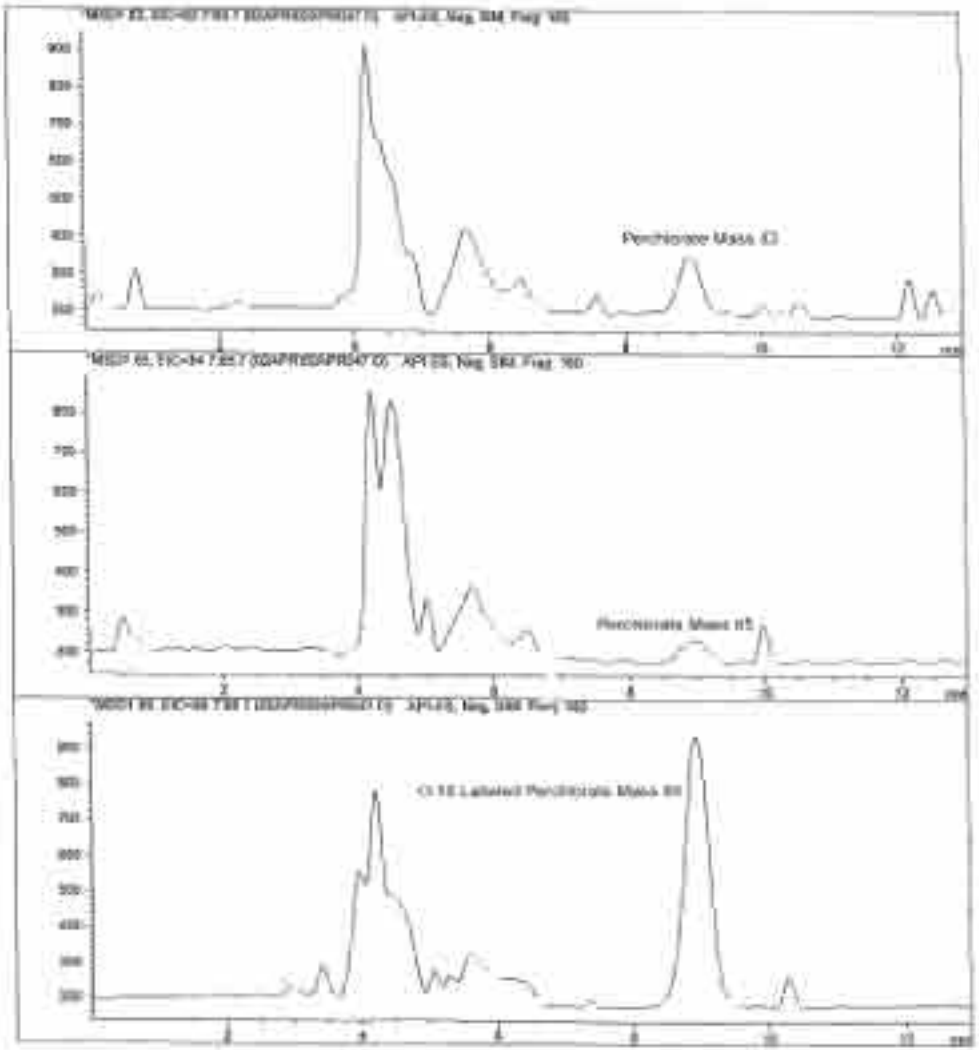
Chromatogram
Great Salt Lake
Matrix

1.0 ug/L



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Perchlorate Detection and Quanitation Levels

Matrix	MDL	PQL
Drinking Water	0.0339 ug/L	0.2 ug/L
Soil	0.811 ug/Kg	2 ug/Kg
Biota (Grass)	1.92 ug/Kg	6 ug/Kg
Simulated Ground Water	0.0807 ug/L	0.2 ug/L
Great Salt Lake	0.0617 ug/L	0.2 ug/L

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Method Validation

- ✓ Calibration
- ✓ Sensitivity
- ✓ Selectivity
- ✓ Precision and Bias
- ✓ Robustness

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Method Validation - Calibration

A minimum of six calibration standards were used for internal standard calibration. Standard concentrations used to calibrate were 0.2, 0.5, 1.0, 2.0, 5.0, and 10.0 µg/L. The internal standard of ^{18}Oxygen Labeled Perchlorate was at 5.0 µg/L. The standard curve for Perchlorate is established by plotting the ratio for each standard/internal standard area against the concentration.

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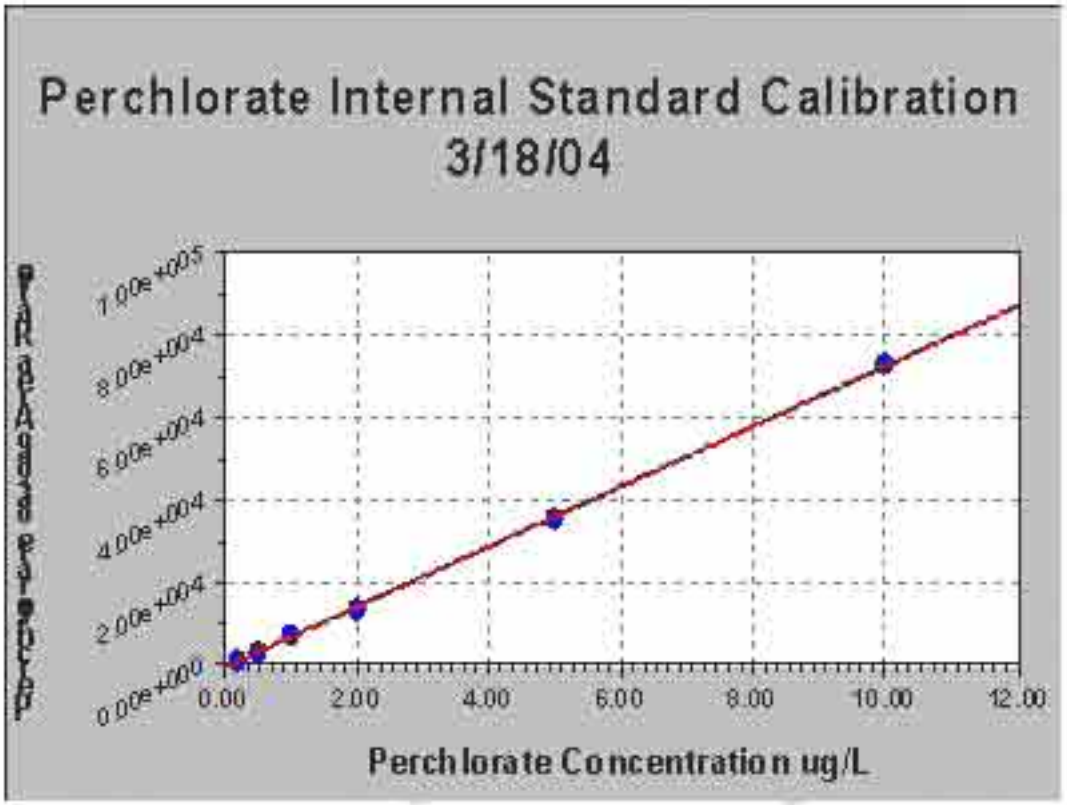
Method Validation - Calibration



Linear Fit: y=a+bx , No weighting used. Correlation Coefficient: 0.9998 Calibration acceptance criterion for the initial calibration curve is a correlation coefficient of 0.995 or higher.

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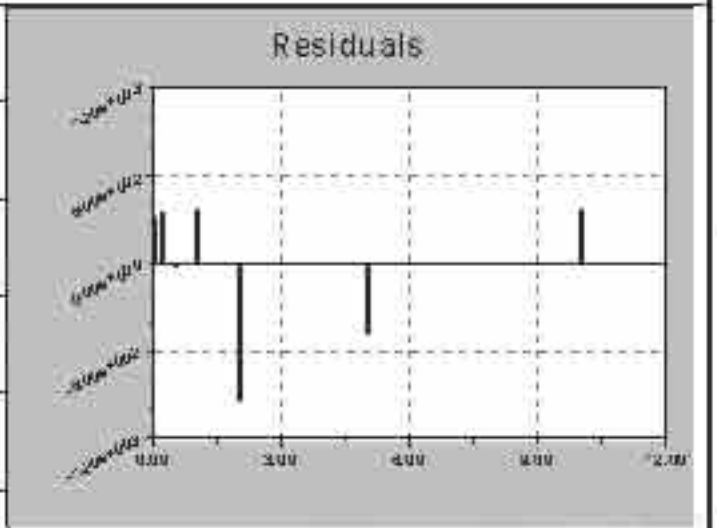
Method Validation - Calibration

Residuals

Concentration	Residual %
0.20	30.5
0.50	0.5
1.0	5.3
2.0	6.5
5.0	1.3
10.0	0.5



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Method Validation - Sensitivity

The MDLs for five matrices were calculated using the procedures specified by the USEPA. Seven aliquots of a fortified spike or indigenous level were analyzed. The MDL is calculated by multiplying the standard deviation of results by 3.143 (*t* statistic). The DW, SGW and Soil samples were spiked with Perchlorate while indigenous levels of Perchlorate in Biota and GSL were used to calculate MDLs.

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Method Validation - Sensitivity

Matrix	n	Units	Spiked Conc	Mean Conc	Standard Deviation	%RSD	Ratio	MDL	PQL
DW	7	ug/L	0.200	0.200	0.0108	5.40%	5.89	0.0339	0.20
Soil	7	ug/Kg	2.00	2.26	0.258	11.4%	2.47	0.811	2.0
Biota (Grass)	7	ug/Kg	4.49	4.49	0.609	13.6%	2.34	1.92	6.0
SGW	7	ug/L	0.200	0.209	0.0257	12.3%	2.48	0.0807	0.20
GSL	7	ug/L	0.219	0.219	0.0196	8.96%	3.55	0.0617	0.20

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Method Validation - Sensitivity

Matrix	MDL Verification Concentration	MDL Verification Result
Drinking Water	0.10 µg/L	0.11 µg/L
Soil	1.0 µg/Kg	1.0 µg/Kg
Biota	2.3 µg/Kg	1.6 µg/Kg
SGW	0.10 µg/L	0.11 µg/L
GSL	0.11 µg/L	0.12 µg/L

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Method Validation - Selectivity

- ✓ Perchlorate 83/85 Isotopic Ratio

- ✓ ^{18}Oxygen Labeled Perchlorate as Internal Standard

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Method Validation - Selectivity

The ratio of 83/85 masses were monitored during this study for all matrices analyzed by this method.

The results of following table and scatter plot shows a lower 83/85 mean ratio at low concentrations of Perchlorate. Based on error of measurement associated with low levels and the importance of confirming Perchlorate the 83/85 isotopic ratio statistical process control limits are set using ± 2 standard deviations at 2.2 to 3.3.

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Method Validation - Selectivity

Mean 83/85 Ratio by Concentration				
Low Concentration	Average:	2.59	Std Dev:	0.28
	LCL[1]:	1.74	UCL[1]:	3.44
Med Concentration	Average:	2.73	Std Dev:	0.32
	LCL[1]:	1.78	UCL[1]:	3.68
High Concentration	Average:	2.89	Std Dev:	0.20
	LCL[1]:	2.27	UCL[1]:	3.50

Total 83/85 Ratio				
Average	Std Dev	n	LCL[2]	UCL[2]
2.75	0.29	121	2.16	3.34

[1] ± 3 SD [2] ± 2 SD

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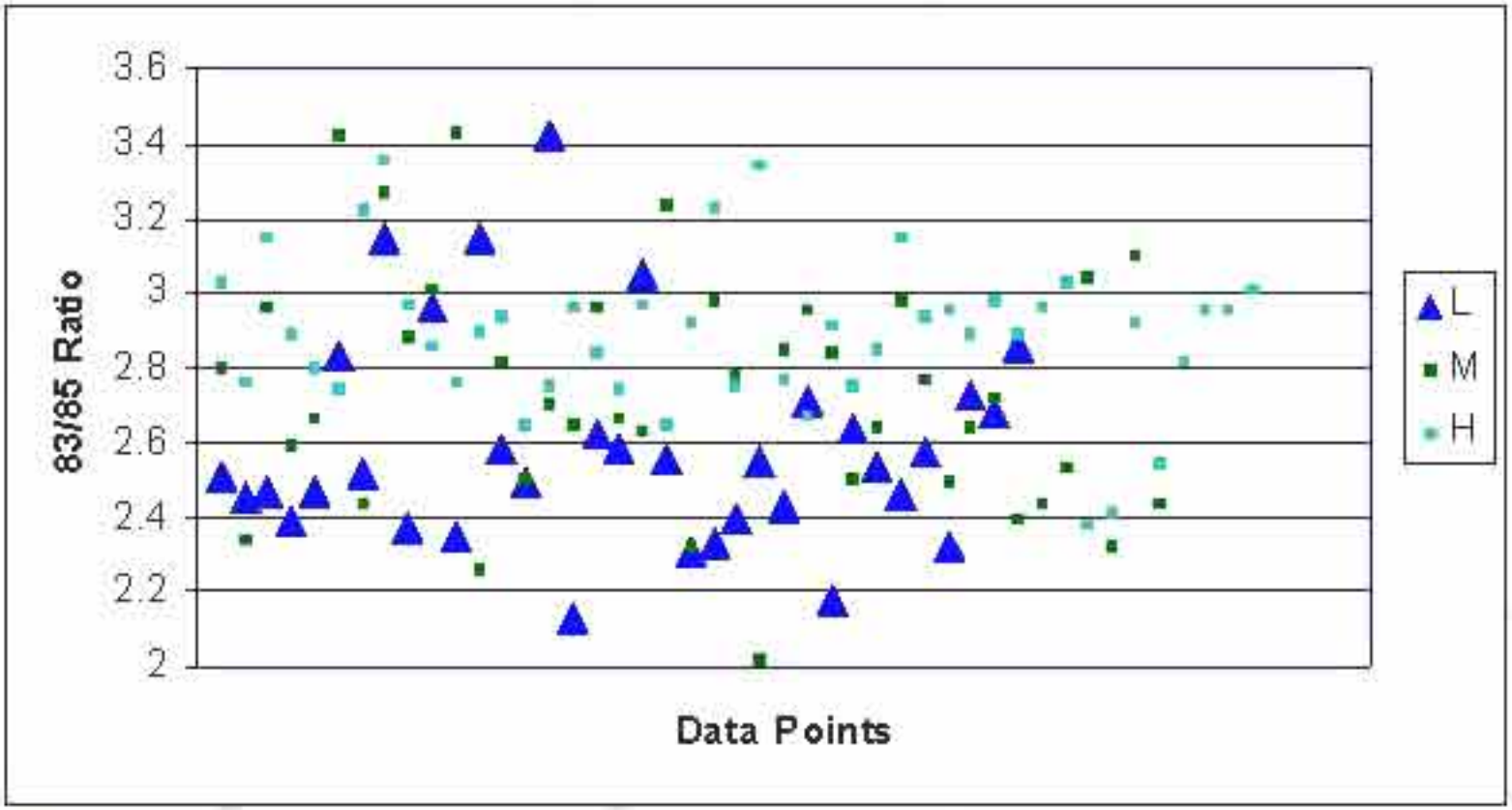
Method Validation - Selectivity



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Method Validation – Precision and Bias

Validation studies for precision and bias were based on NELAC 2003 Chapter 5 were generated for five matrices by analyzing samples over three consecutive days at varying concentration levels. The study analyzed nine replicates for each matrix on a daily basis. The three concentrations are at or near the limit of quantitation, at the upper-range of the calibration (upper 20%) and at a mid-range concentration.

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Method Validation – Precision and Bias

To compare the variability of performance (precision) the *F*-Test was performed on each matrix. Matrices were evaluated based on concentration levels, combined daily results and used to compare the precision of this method on the five matrices.

$$F = \frac{(RSD_X)^2}{(RSD_Y)^2}$$

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Method Validation – Precision and Bias

If F > 0.17 and F < 6.03, then the variability of performance for this method with respect to concentrations in the same matrix are not different.

Matrix	Low vs. Med	Low vs. High	Med vs. High
Drinking Water	2.86	8.05	2.81
Soil	1.18	3.52	2.98
Biota	0.38	1.88	4.98
SGW	2.70	9.79	3.62
GSL	0.71	2.15	3.03

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Method Validation – Precision and Bias

If F > 0.17 and F < 6.03, then the variability of performance for this method with respect to daily analysis for all concentrations in the same matrix are not different.

Matrix	Day 1 vs. Day 2	Day 1 vs. Day 3	Day 2 vs. Day 3
Drinking Water	1.89	1.89	1.00
Soil	1.16	2.04	1.75
Biota	0.41	0.65	1.60
SGW	0.60	0.92	1.53
GSL	1.69	0.67	0.40

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Method Validation – Precision and Bias

If F > 0.40 and F < 2.55, then the variability of performance for this method with respect to matrix for all concentrations on all days are not different.

Matrix / Matrix	Soil	Biota	SGW	GSL
Drinking Water	1.46	0.95	0.51	0.69

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Method Validation – Precision and Bias

The evaluation of method bias was accomplished by:

✓ Proficiency Testing

✓ To compare the variability of means of each aqueous matrix the Paired *t*-Test was used

✓ Comparision of sample analysis by 314.0

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Method Validation – Precision and Bias

PT Study	Result 314.0	Result LC/MS	True Value
WS04-1	47.3 µg/L	51.2 µg/L	52.7 µg/L
Potable WatR™ Perchlorate 052004A	NA	5.64 µg/L	5.48 µg/L
WS04-3	89.7 µg/L	83.8 µg/L	90.0 µg/L

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Method Validation – Precision and Bias

Comparison of the variability of means of each aqueous matrix using the Paired *t*-Test.d

If |t| < 2.479, the variability of means of each aqueous matrix with respect to this method are not significantly different.

$$t = Mean\,Difference_{MatrixX-MatrixY} \times \frac{\sqrt{n}}{Stdev\,Difference_{MatrixX-MatrixY}}$$

Matrix:	DW vs. SGW	DW vs. GSL	SGW vs. GSL
|t|	1.74	0.51	2.07

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Method Validation – Precision and Bias

Sample Matrix	Result by 314.0	Result by LC/MS	Confirmation Achieved
Water 04C00326	0.76 µg/L(J)	0.87 µg/L	Yes
Water 04C00327	0.87 µg/L(J)	1.1 µg/L	Yes
Water 04C00328	1.8 µg/L	1.8 µg/L	Yes
Water 04C00329	1.6 µg/L	1.8 µg/L	Yes
Water 04C00330	1.6 µg/L	1.4 µg/L	Yes
Water 04C00331	1.2 µg/L	1.5 µg/L	Yes
Water 04E02488	0.36 µg/L(J)	0.40 µg/L	Yes
Water 04E01966	0.40 µg/L(J)	0.41 µg/L	Yes
Water 04C00732	1.1 µg/L	1.4 µg/L	Yes
Water 04C00736	1.4 µg/L	1.6 µg/L	Yes
Water 04C00737	1.1 µg/L	1.4 µg/L	Yes
Soil 04C00678	6.7 µg/Kg	4.5 µg/Kg	Yes
Soil 04C00680	8.9 µg/Kg	ND	No

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Method Validation - Robustness

✓ A single calibration curve was used for this entire study. Results of CCV analysis during the validation study are used to assess the stability of the instrument calibration. Calibration is verified if the relative percent difference is less than 15%.

✓ Use of ^{18}Oxygen Labeled Perchlorate as an internal standard has reduced calibration runs and eliminates worrisome variation in the mass spectrometer due to matrix interferences. The internal standard area counts are monitored and must be within ± 30% of the daily calibration verification response.

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Method Validation - Robustness

Date/Time	Result	Nominal Value	% Difference
4/2/04 4:29 PM	10.45	10.0	4.5%
4/2/04 7:16 PM	1.005	1.0	0.5%
4/2/04 9:48 PM	9.25	10.0	7.6%
4/3/04 5:24 AM	0.998	1.0	0.2%
4/3/04 11:52 AM	10.45	10.0	4.5%
4/3/04 2:40 PM	0.949	1.0	5.1%
4/3/04 5:12 PM	10.51	10.0	5.1%
4/3/04 7:44 PM	0.989	1.0	1.1%
4/3/04 10:16 PM	10.66	10.0	6.6%
4/4/04 9:52 AM	11.008	10.0	10.1%
4/4/04 12:39 PM	1.027	1.0	2.7%
4/4/04 3:11 PM	10.14	10.0	1.4%
4/4/04 5:43 PM	0.983	1.0	1.7%
4/4/04 8:15 PM	10.52	10.0	5.2%
4/4/04 10:47 PM	1.015	1.0	1.5%

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LCS Control Limits

Matrix	Mean Recovery	Standard Deviation	Lower Control Limit (LCL)	Upper Control Limit (UCL)
DW	103.9%	7.8%	80.5%	127.2%
Soil	102.9%	6.3%	83.8%	121.9%
Biota	105.9%	8.0%	81.8%	130.1%
SGW	98.9%	10.4%	67.6%	130.2%
GSL	104.8%	9.3%	76.9%	132.6%
All Matrices	103.3%	8.7%	77.2%	129.4%

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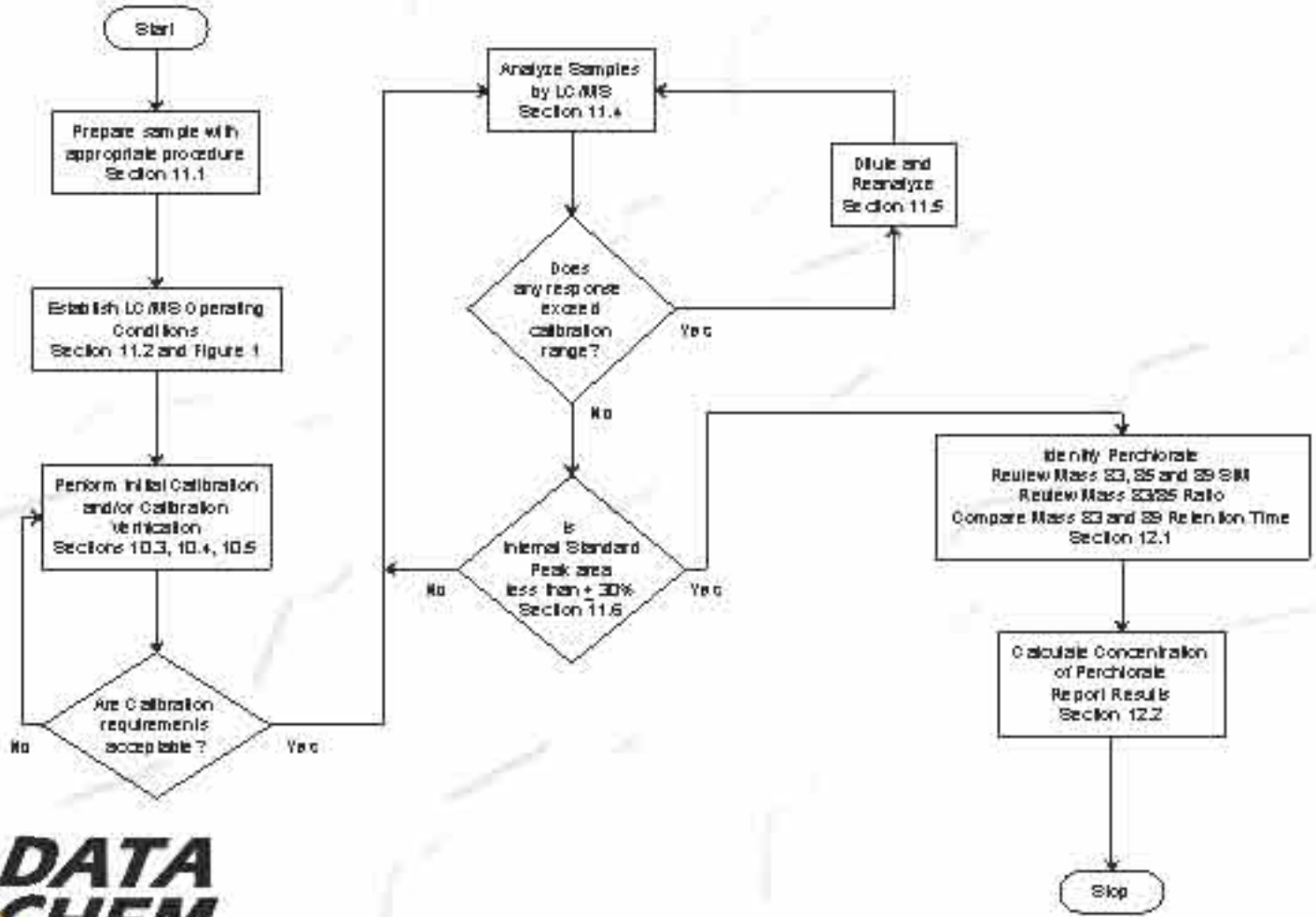
6850 Quality Control



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6850 Quality Control

- ✓ 9.4 Initial Demonstration of Proficiency
- ✓ 9.6.1 Method Blank in each batch (<1/2 RL)
- ✓ 9.6.2 LCS in each batch (80% to 120%)

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6850 Quality Control

- ✓ 9.7.1 Matrix Spike in each batch (80% to 120%)
- ✓ 9.7.2 Matrix Spike Duplicate in each batch (± 15%)
- ✓ 9.9 Method Sensitivity (MRL ≥ Low Calibration Point)

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6850 Quality Control

- ✓ 10.3 Initial Calibration (Correlation Coefficient $\geq$ 0.995)
- ✓ 10.4 Initial Calibration Verification

  (Second Source and ± 15%)
- ✓ 10.5 Continuing Calibration Verification

  (Every 10 samples and at end of run)

  (± 15%)

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- ✓ 11.5 Dilute samples above calibration range.
- ✓ 11.6 Sample Internal Standard Peak Area

  (± 30% from daily ICV or CCV)

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6850 Quality Control

✓ 12.1 Perchlorate Identification

Retention Time of Internal Standard

83/85 Isotopic Ratio (2.2 to 3.3)

83/89 Internal Standard Calibration

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6850 Future Plans

- Comments are being collected and will be addressed in draft 6850 method by December 2004

- 6850 will be submitted to the USEPA OSW Inorganic Method Workgroup by January, 2005

- Multi-laboratory Validation Study – early 2005

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Thank You

Questions?

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