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Harnessing the Power of Mass Spectrometry and Automation to Reduce Sample Size, Sample Preparation Time and Increase Laboratory Efficiency

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Method 1633 for 40 PFAS Compounds

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EPA's Office of Water, in partnership with the Department of Defense's (DoD) Strategic Environmental Research and Development Program, has published Method 1633, a method to test for **40 PFAS compounds in wastewater, surface water, groundwater, soil, biosolids, sediment, landfill leachate, and fish tissue.**
Link to website

Intended to be used as follows with Regulations in development in US:

- Military sites
- Clean Water Act Compliance (wastewater discharge permits)
- Superfund sites
- General remediation and investigation programs

EPA method 1633

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Adjusting the Method

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Step	1633 as written	Changes
SPE extraction	500 mL sample volume manual extraction using negative pressure manifold • Time consuming and ties up scientist	**Automated SPE system** • Allows scientists to spend time on other responsibilities 
SPE cartridge	Weak Anion Exchange (WAX) SPE followed by dispersive Graphitized Carbon Black (GCB) clean-up • GCB difficult to work with	**Dual phase SPE cartridge containing WAX and GCB sorbents** • Allows sample extraction and clean-up steps to be fully automated 
LC-MS/MS analysis	Tandem quadrupole analysis for sensitivity and selectivity	**High sensitivity tandem quad mass spectrometer** • Allows for reduction of sample size 

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Fully automated SPE and clean-up method

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1.
   - Spike water sample with Extracted Internal Standard Mix (MPFAC-HIF-ES from Wellington)
   - Check pH and adjust to approximately 6 if necessary
2.
   - Condition SPE cartridges
     - 15 mL 1% ammonium hydroxide in methanol
     - 5 mL 0.3 M formic acid
3.
   - Load sample at 5 mL/min
   - Wash cartridge with 10 mL of reagent water – bottle rinse with solution
   - Wash with 5 mL of 1:1 0.1M formic acid:methanol – bottle rinse with solution
   - Dry cartridge for 1 minute
   - Elute with 5 mL 1% ammonium hydroxide in methanol - bottle rinse with solution
4.
   - Add 25 µL acetic acid to each sample
   - Spike each sample with Non Extracted Internal Standard (MPFAC-HIF-IS from Wellington)

Promochrom SPE-03 MOD04

Oasis WAX/GCB for PFAS Analysis Bilayer Dual Phase Cartrdige

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- Reduced sample size from 250 mL to 50 mL
  - Ground water
  - Surface water
  - Influent Wastewater
  - Effluent Wastewater

Sample Volume	Manual Time	Automated Time
250 mL	~ 3-4 hours (10 samples)	~ 2.25 hours (8 samples)
50 mL	-	~ 1 hour (8 samples)

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EPA 1633 Instrument Methods

Source Parameters

- Instrument: Xevo TQ Absolute MS
- Ion Mode: ESI-
- Capillary Voltage: 0.5 kV
- Desolvation Temperature: 350°C
- Desolvation Flow: 900 L/hr
- Cone Flow: 150 L/hr

LC Method

- Instrument: ACQUITY™ Premier BSM FTN System with PFAS Kit
- Column: ACQUITY Premier **BEH™ C18** Column 2.1mm x **50 mm**, 1.7 µm
- Isolator Column: Atlantis™ Premier **BEH C18 AX** Column 2.1mm x 50 mm, 5.0 µm
- Mobile Phase A: Water + 2 mM ammonium acetate
- Mobile Phase B: **Acetonitrile** + 2 mM ammonium acetate
- Injection Volume: 10 uL
- **Gradient:**

Time (min)	Flow (mL/min)	%A	%B
0	0.3	95	5
0.5	0.3	75	25
3	0.3	50	50
6.5	0.3	15	85
7	0.3	5	95
8.5	0.3	5	95
9	0.3	95	5
11	0.3	95	5

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Quality Control Parameters of LC-MS/MS Method

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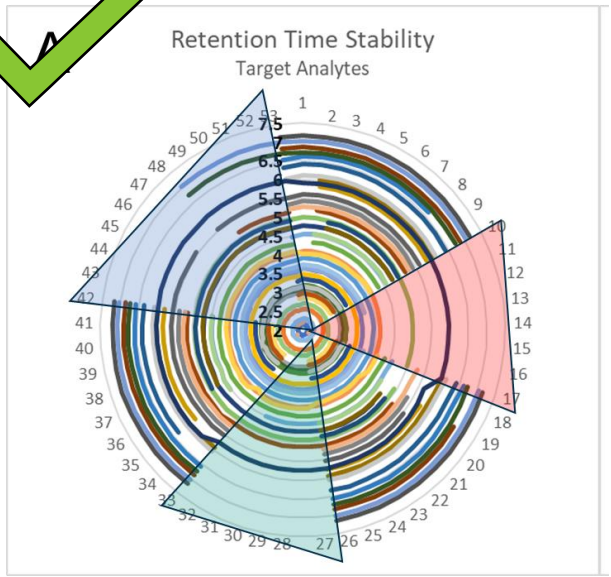

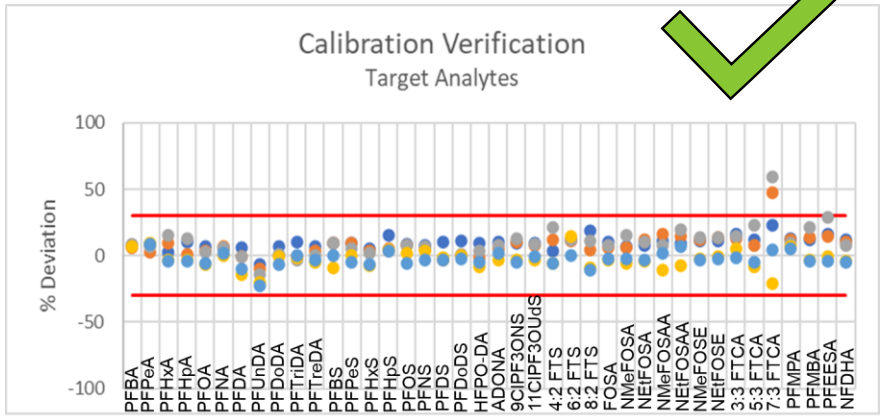

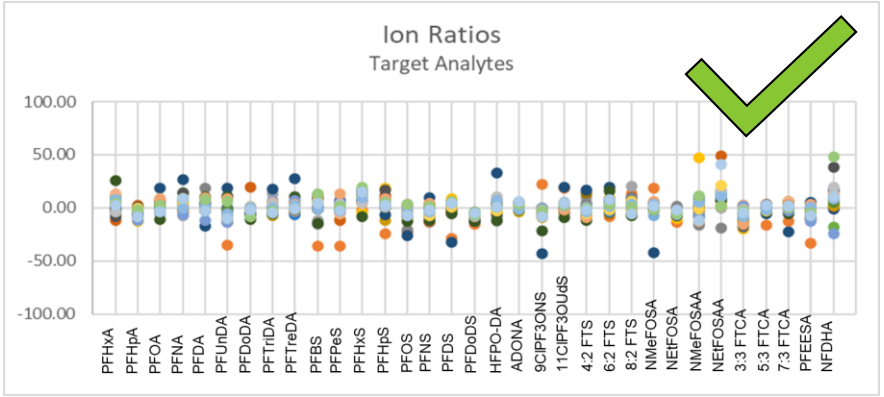

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Method Detection Limits

- n = 8 samples used to calculate MDLs in 50 mL extracts
- Blanks and samples extracted and analyzed over 3 day period
- MDLs for 50 mL sample size equivalent or lower than those reported in EPA 1633 method using 500 mL sample

Compound	50 mL sample MDL ng/L	EPA 1633 MDL ng/L	Compound	50 mL sample MDL ng/L	EPA 1633 MDL ng/L
PFBA*	1.00	0.79	ADONA	0.34	0.50
PFPeA*	0.53	0.54	NFDHA	0.42	0.75
PFHxA	0.14	0.46	9ClPF3ONS	0.33	1.38
PFHpA	0.24	0.37	11ClPF3OUdS	0.28	1.67
PFOA*	0.41	0.54	4:2 FTS	0.87	1.69
PFNA*	0.85	0.45	6:2 FTS	0.43	2.45
PFDA	0.13	0.52	8:2 FTS	0.80	2.50
PFUnDA	0.23	0.45	PFOSA	0.17	0.32
PFDoDA	0.15	0.40	NMeFOSA	0.25	0.43
PFTriDA	0.28	0.46	NEtFOSA	0.18	0.45
PFTreDA	0.18	0.49	NMeFOSAA	0.16	0.68
PFBS	0.09	0.37	NEtFOSAA	0.18	0.59
PFPeS	0.18	0.50	NMeFOSE	1.79	3.81
PFHxS	0.16	0.54	NEtFOSE	1.24	4.84
PFHpS	0.15	0.50	3:3 FTCA	0.86	2.47
PFOS	0.11	0.63	5:3 FTCA	1.63	9.59
PFNS	0.18	0.47	7:3 FTCA	2.33	8.71
PFDS	0.23	0.60	PFMPA	0.26	1.46
PFDoDS	0.13	0.60	PFMBA	0.19	1.41
HFPO-DA	0.43	0.51	PFEESA	0.19	1.17

* Indicates the MDL is calculated taking into account blank contamination for this compound

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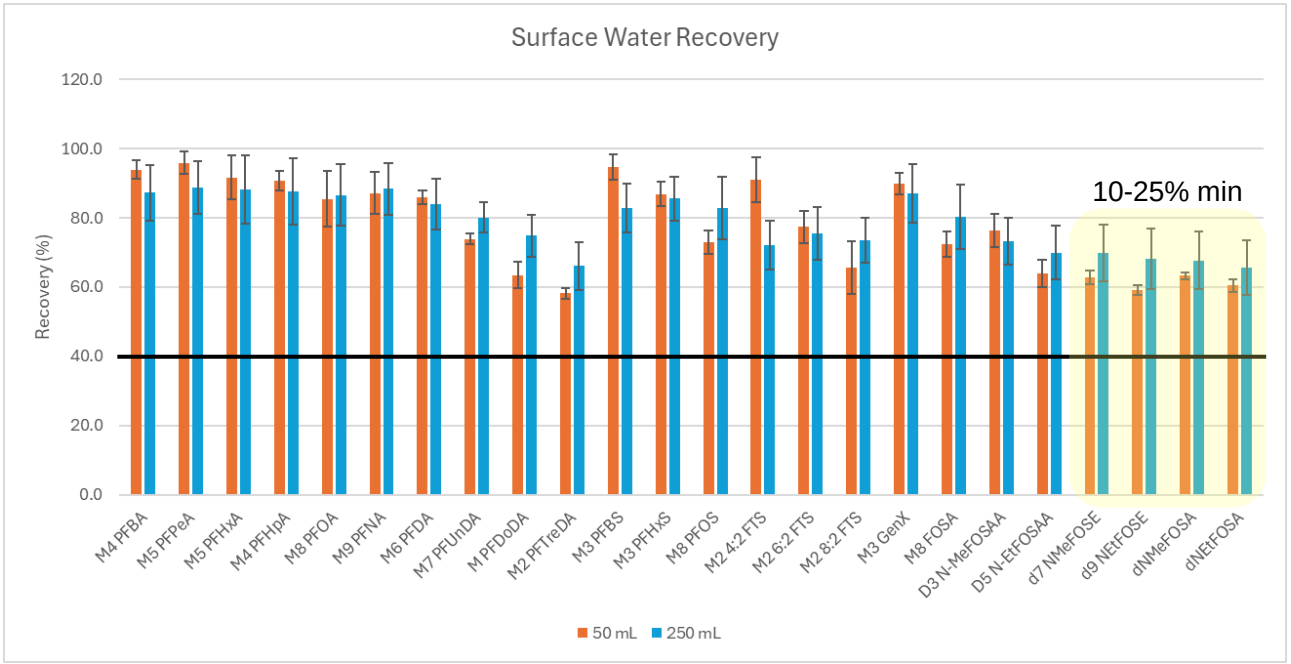
Recovery in 50 mL water samples



- Recovery of extracted internal standards in 50 mL surface water samples (with TSS) was equivalent to recovery in 250 mL sample size extractions

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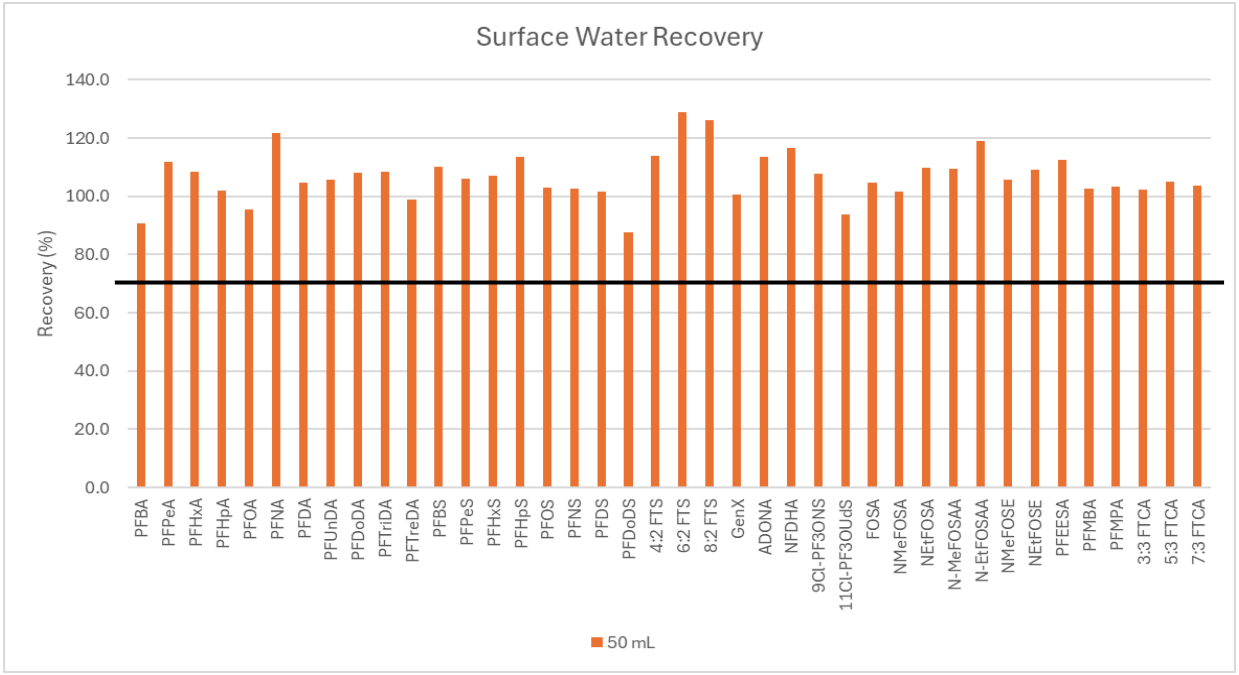
Recovery in 50 mL water samples



- Recovery of natives in 50 mL surface water samples (with TSS) was well within EPA 1633 acceptance limits

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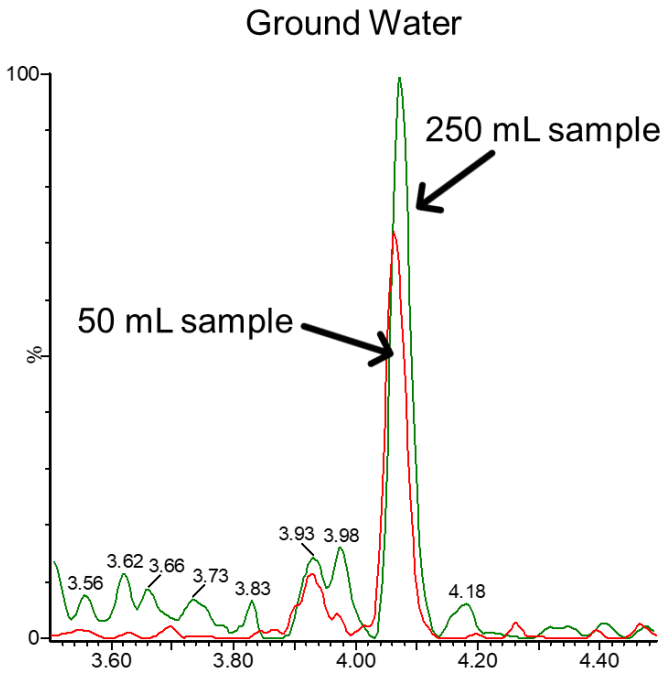
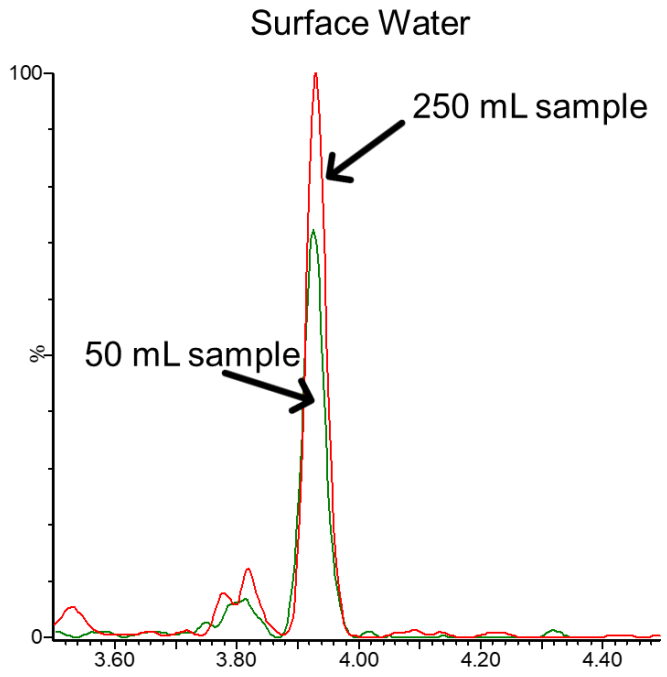
Sensitivity Comparison

- 2 µL injection volume for 250 mL sample extracts
- 10 µL injection volume for 50 mL sample extracts
- Peak response for PFHxS in ground and surface water is within ~75% of 250 mL sample





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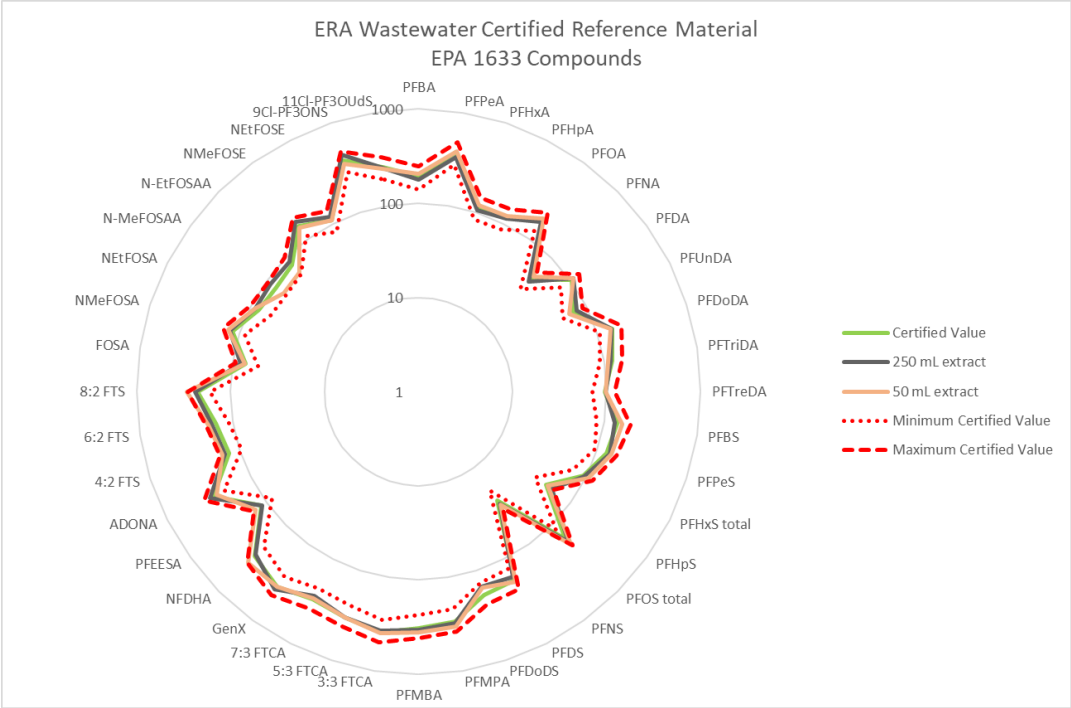
Method Check –Certified Reference Materials

- Wastewater certified reference material extract
  - Prepared in 50 and 250 mL size
- Equal performance of CRM using 250 mL and 50 mL extract size



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PFAS Detected in Water Samples

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- PFAS were detected in all water sample types
  - 9/40 detected in ground water
  - 11/40 detected in surface water
  - 20/40 detected in influent water
  - 22/40 detected in effluent water
- Range of concentrations detected from 0.1 – 90.1 ng/L
  - Approximately a 1000-fold difference

Compound	Ground Water (ng/L)	Surface Water (ng/L)	Influent Wastewater (ng/L)	Effluent Wastewater (ng/L)
PFBA	4.9	2.7	12.7	12.0
PFPeA	2.0	2.9	24.0	11.2
PFHxA	2.7	4.5	90.1	23.4
PFHpA	1.3	3.1	5.4	4.6
PFOA	2.0	7.4	15.2	10.9
PFNA	2.1	3.8	4.4	3.5
PFDA	N.D.	1.2	3.3	1.6
PFUnDA	N.D.	0.6	0.8	0.8
PFDoDA	N.D.	N.D.	0.7	0.5
PFTriDA	N.D.	N.D.	0.3	0.2
PFTreDA	N.D.	N.D.	0.2	0.1
PFBS	1.4	3.2	36.2	32.4
PFHxS Total	0.3	1.2	8.1	8.8
PFHpS	N.D.	N.D.	0.2	N.D.
PFOS Total	0.1	7.6	10.9	12.7
6_2 FTS	N.D.	N.D.	6.3	10.9
FOSA	N.D.	N.D.	0.3	0.4
NMeFOSAA	N.D.	N.D.	1.5	2.0
NEtFOSAA	N.D.	N.D.	0.6	1.6
NMeFOSE	N.D.	N.D.	N.D.	6.2
NEtFOSE	N.D.	N.D.	N.D.	4.8
5:3 FTCA	N.D.	N.D.	5.3	48.9
7:3 FTCA	N.D.	N.D.	N.D.	6.1

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Conclusions

- Simple adjustments made to LC-MS/MS method
- Using a highly sensitive tandem quadrupole mass spectrometer allows sample volume reduction for EPA 1633 from 500 mL to 50 mL (**and below?**)
  - Faster sample loading and overall sample preparation time
  - Less loading of TSS/particulates on SPE cartridges creates less chance for cartridge blocking issues
  - Equal performance to larger volume size samples
- Automated sample preparation increases throughput and efficiency of the lab
  - Full automation is possible using a WAX cartridge with the GCB packed in it
- All EPA 1633 quality control guidelines met with method

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Acknowledgements

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