Collaborative Efforts to Develop an EPA Method for 6PPD-Q in Aqueous Matrices

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The presenter is *not* an EPA employee, but a contractor.

What is 6PPD-Q?

6-phenylenediamine quinone, a.k.a.:

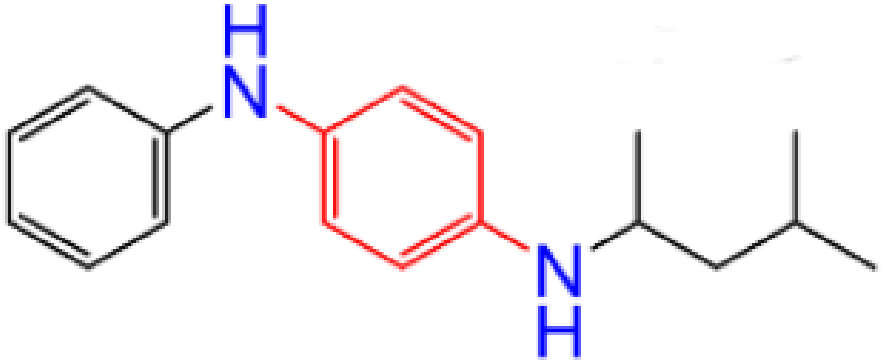
- 6PPD-Q,
- *p*-phenylenediamine quinone,
- 2-anilo-5-[(4-methylpentan-2-yl)amino]cyclohexa-2,5-diene-1,4-dione,
- N-(1,3-Dimethylbutyl)-N′-phenyl-*p*-phenylene diamine-quinone, or
- 2-[(1,3-Dimethylbutyl)amino]-5-(phenylamino) 2,5-cyclohexadiene-1,4-dione

Formed by the oxidation of *p*-phenylenediamine, or 6PPD (an antioxidant added to tires), with ozone, and released to the environment as tires wear out

6PPD also found in other rubber products such as footwear, synthetic turf infill, and synthetic playground surfaces

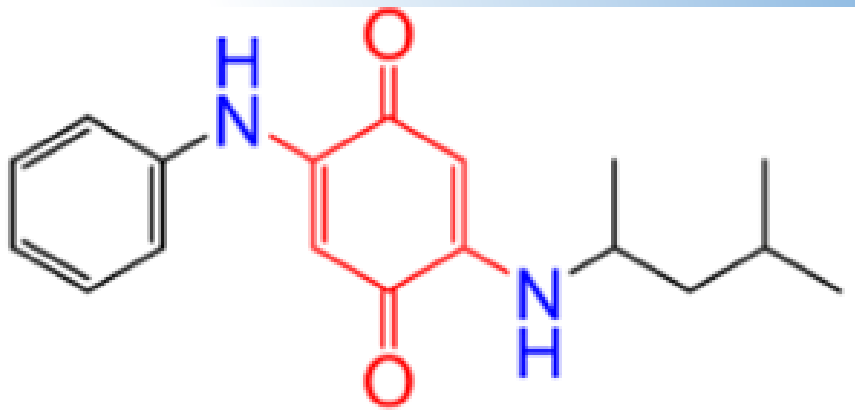
Structures

6PPD



+ Ozone →

6PPD-Q



Why Do We Care?

- Coho salmon (*Oncorhynchus kisutch*) are an anadromous species that migrate from the ocean to freshwater to spawn
- Coho salmon are a culturally and commercially important species in the Northwest U.S.
- A December 2020 publication by the University of Washington reported that 6PPD-Q was found in stormwaters and Pacific NW rivers after rainfall events at concentrations toxic to Coho salmon (Tian *et al.*, 2020, *Science* 10.1126/science.abd6951)
- The study observed 6PPD-Q at 0.3 to 19 µg/L in roadway runoff and stormwater-influenced surface waters
- LC_{50} for Coho salmon has been estimated to be 95 ng/L (0.095 µg/L)
- The Nisqually tribe formally requested that EPA “develop analytical tools and methods to measure and regulate 6PPPD in stormwater”

EPA Region 10's Response

- Early in 2021, EPA Region 10 convened a monthly roundtable of federal, state and tribal scientists
- The group expressed a strong need for an EPA method for this contaminant in surface waters and stormwaters
- Region 10 initiated a collaborative effort with Eurofins-Sacramento to conduct a single-laboratory validation study of an existing Eurofins LC-MS/MS method that utilized SPE and isotope dilution quantitation

Early Single-laboratory Efforts

- Shortly after the December 2020 article in *Science*, Eurofins Sacramento began to investigate 6PPD-Q, using high resolution MS
- Water from a parking lot storm drain was analyzed and a peak at the exact mass of 6PPD-q was identified, along with two robust daughter fragments identified in MS/MS mode
- Authentic 6PPD-Q standards were commercially available by Spring 2021, along with the deuterated analog (D_5-6PPD-Q)
- Early Eurofins data demonstrated the affinity of 6PPD-Q for suspended material, which ruled out a direct injection method

Single-laboratory Validation Study

- Region 10 sought input from the Office of Water to ensure that the study would be rigorous enough to support development of a formal EPA method in the 1600-Series
- In collaboration with EPA Region 10, a single-laboratory validation study was designed and formalized in a study plan in July 2022
- The SLVS focused on development of SPE and LC/MS/MS procedures, including the initial demonstration of capabilities, method detection limit, and storge stability studies
- The matrix types of interest limited to stormwater and surface water to start (wastewater to be done later)

Region 10 and OW Collaboration

- SLVS data reviewed by Region 10 in July 2023
- Region 10 provided the SLVS results and the Eurofins SOP to OW in August 2023
- SLVS study report prepared by Eurofins in Nov. 2023
- GDIT reviewed the results under contract to OW
- GDIT drafted method for OW based on the SOP
- Draft method underwent review - Sept. to Dec. 2023
- OW released Draft Method 1634 on January 30, 2024, in what the Region 10 Administrator termed “lightspeed”

Draft Method 1634

- Applicable to aqueous matrices
- 250-mL sample aliquot is spiked with $^{13}C_6$-6PPD-Q solution as an extracted internal standard (EIS)
- Solid-phase extraction using a polymeric reversed-phase sorbent (Phenomenex Strata-XL)
- Elution with acetonitrile
- Extract is spiked with D_5-6PPD-Q solution as a non-extracted internal standard (NIS)
- Analysis by LC/MS/MS on a C_{18} column, detected using multiple reaction monitoring (MRM), and quantified by isotope dilution

Draft Method 1634 (continued)

- **Injection volume:** 20 µL
- **LC mobile phases:** 0.2% Formic acid in water and acetonitrile
- **Gradient:** 90/10% to 10/90% over a 10-minute run time

Analyte	MRM Transitions
6PPD-Q	299.2 > 215.1 (quantitation ion)
6PPD-Q	299.2 > 241.1 (confirmation ion)
$^{13}C_6$-6PPD-Q (EIS)	305.2 > 221.1
D_5-6PPD-Q (NIS)	304.2 > 220.1

Example Single-Lab Performance Data

IPR Study #	Mean Rec. (%) (n=4)	RSD (%)
1	89.8	2.0
2	90.0	3.4
3	89.3	3.0
4	88.8	1.8
5	88.8	2.2

More Example Single-Lab Performance Data

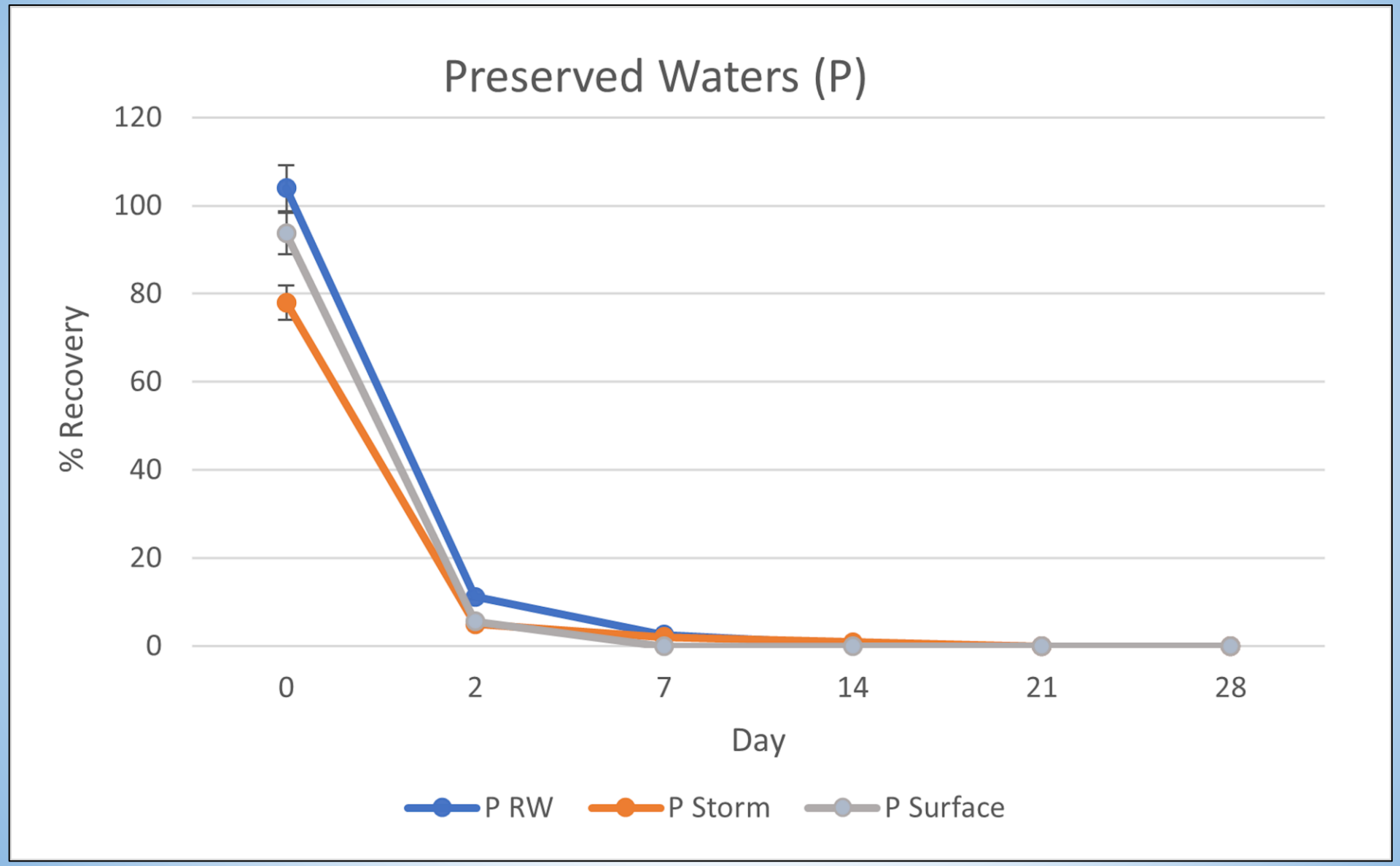
OPR Data			
n	Mean Recovery (%)	RSD (%)	Range (%)
20	89.3	2.7	85 - 92

Pooled MDL_s and ML values - 3 MDL Studies	
Pooled MDL_s	ML
0.43 ng/L	2.0 ng/L

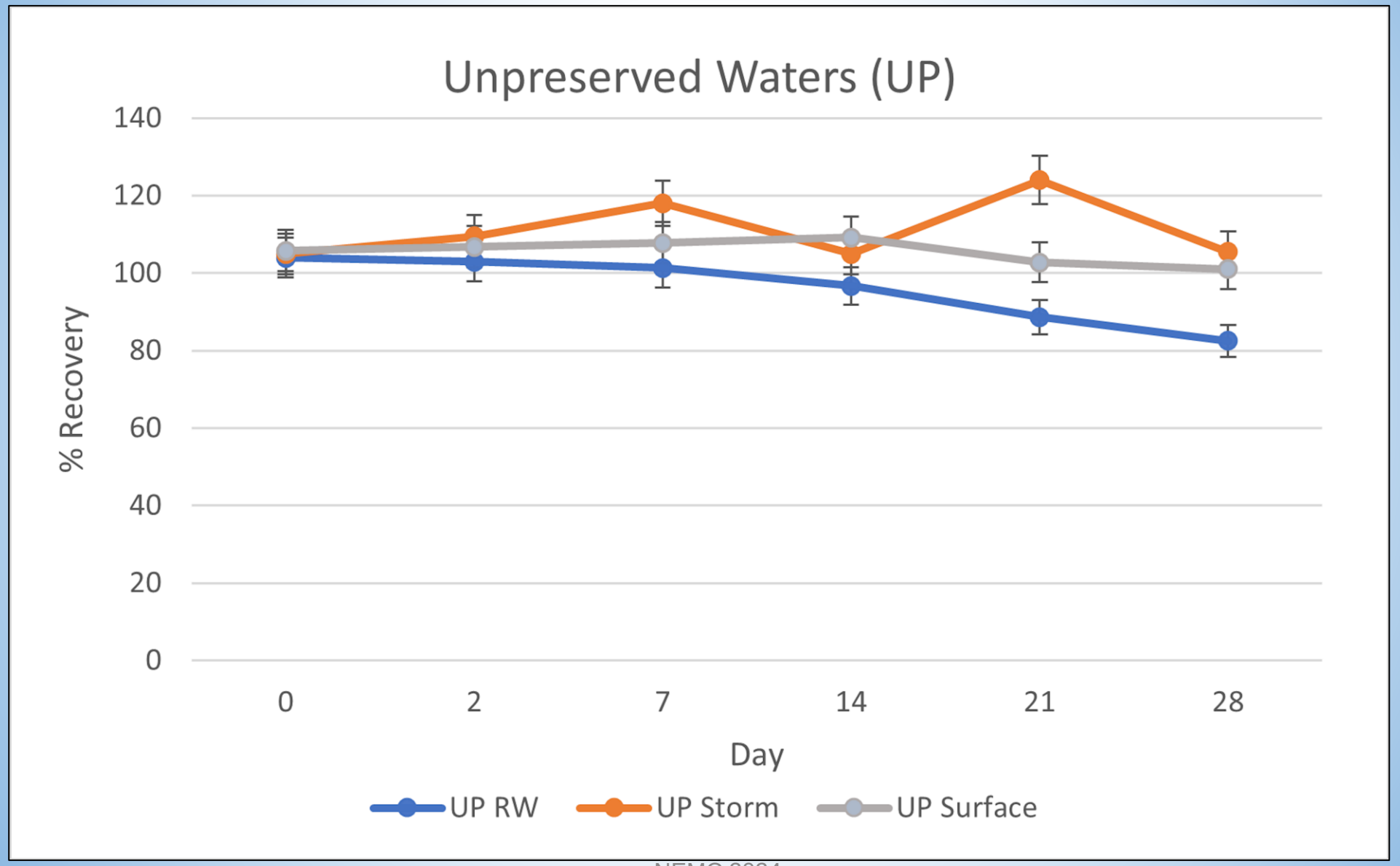
Preservation Studies

- Early work by Eurofins and USGS showed that samples need to be collected in amber glass containers with minimal headspace
- In addition to cold storage at 0-6 °C, a combination of two preservative agents was tested: sodium bisulfate ($NaHSO_4$ at 1 g/L) and sodium metabisulfite ($Na_2S_2O_5$ at 50 mg/L)
  - Sodium bisulfate lowers the pH to roughly 3
  - Sodium metabisulfite rapidly forms sodium sulfite in water, which is an ozone scavenger
- Triplicate analyses at Days 0, 2, 7, 14, 21, 28, and 35
- Study results demonstrate that these agents did *not* aid in preserving spiked 6PPD-Q, but rather appeared to accelerate the degradation/loss of 6PPD-Q

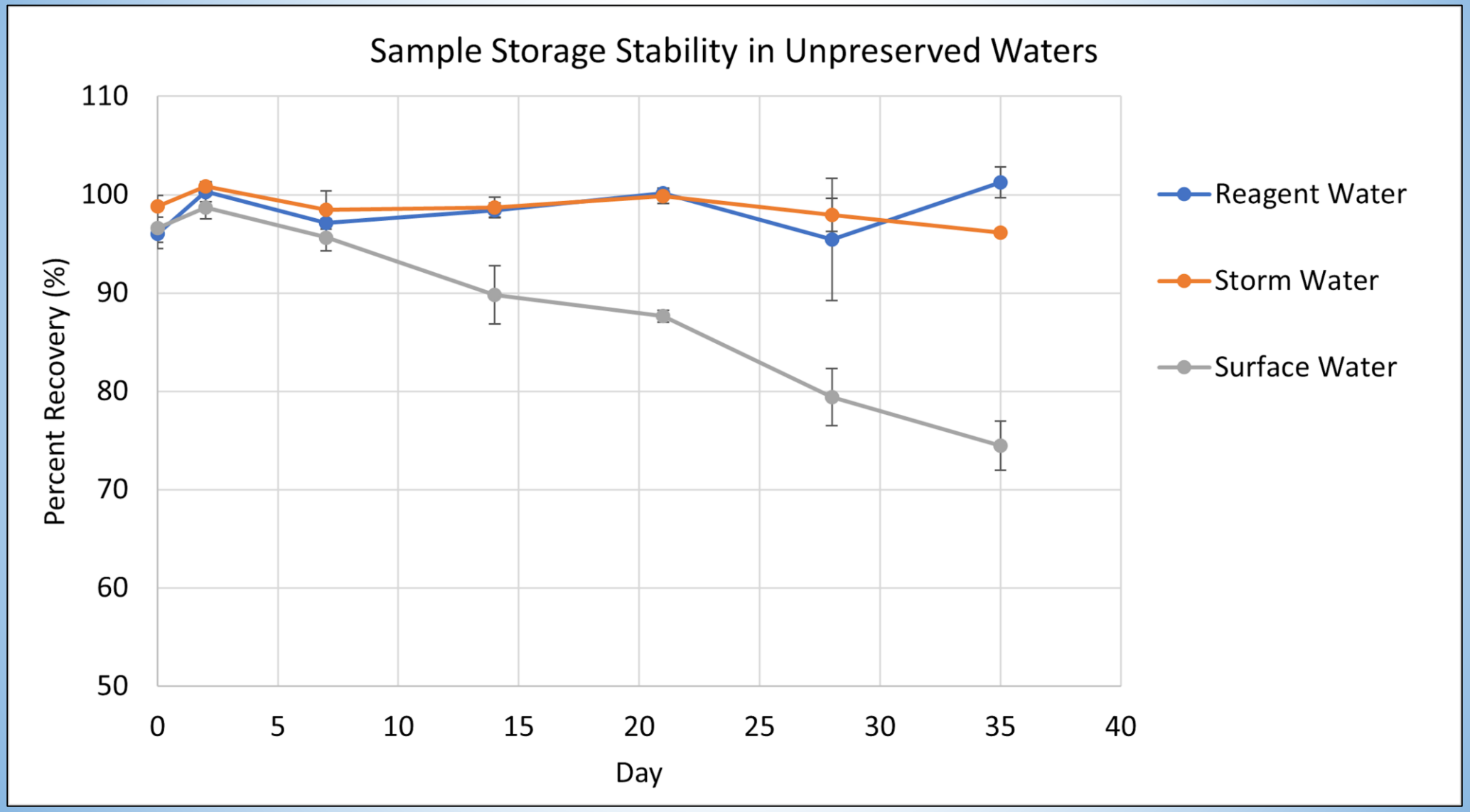
Storage Stability Results for Preserved Waters



Storage Stability – Unpreserved Results



2nd Stability Study - Unpreserved Samples



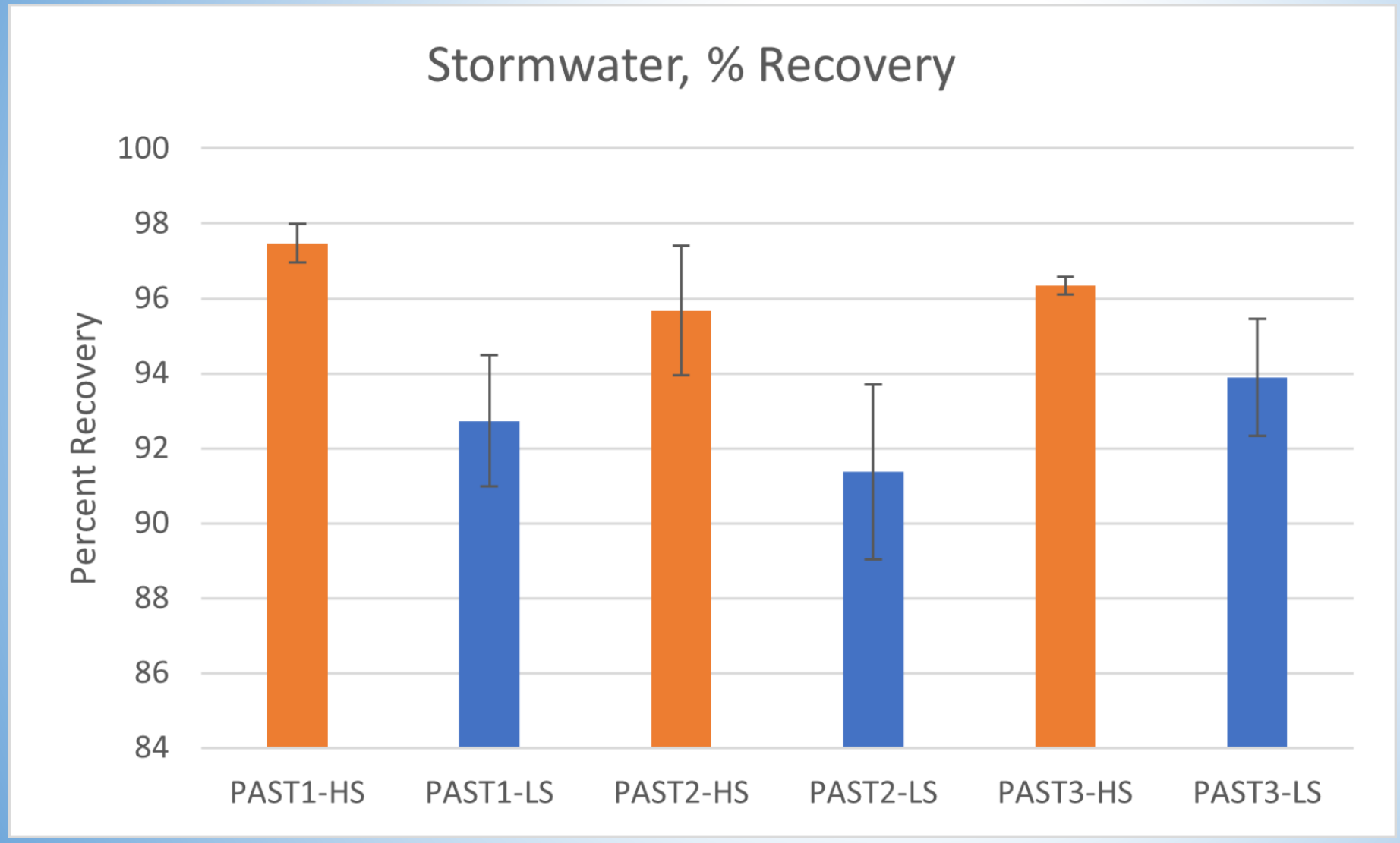
Preservation and Holding Time Decisions

- Based on these results, Draft Method 1634 does *not* employ a preservative, but requires:
  - Samples be collected in amber glass bottles with minimal headspace
  - Storage at 0 – 6 ºC (but keep from freezing) and protected from light
- Aqueous samples must be extracted within 14 days from sample collection
- Extracts must be stored at 0 – 6 °C and analyzed within 28 days from extraction

Method Performance in Stormwaters and Surface Waters Spiked at 2 Concentrations (n=4)

Matrix	Spike (ng/L)	Mean % Recovery	% RSD
Storm	20	92.7	2.45%
Storm	200	96.5	1.39%
Surface	200	94.7	1.76%
All Samples and Matrices (48)		95.2	2.95%

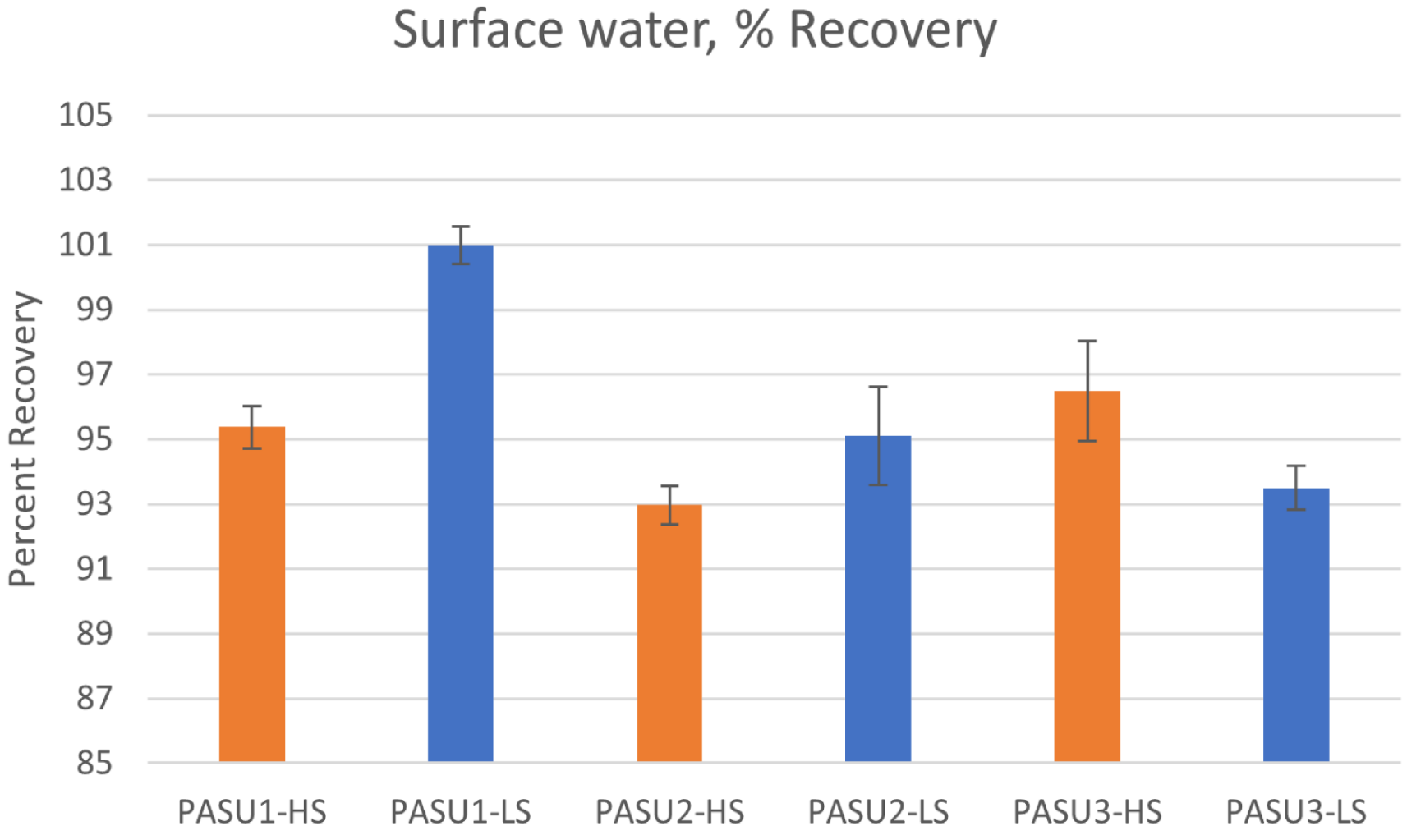
Precision and Accuracy in Stormwater



HS = High spike = 200 ppt

LS = Low spike = 20 ppt

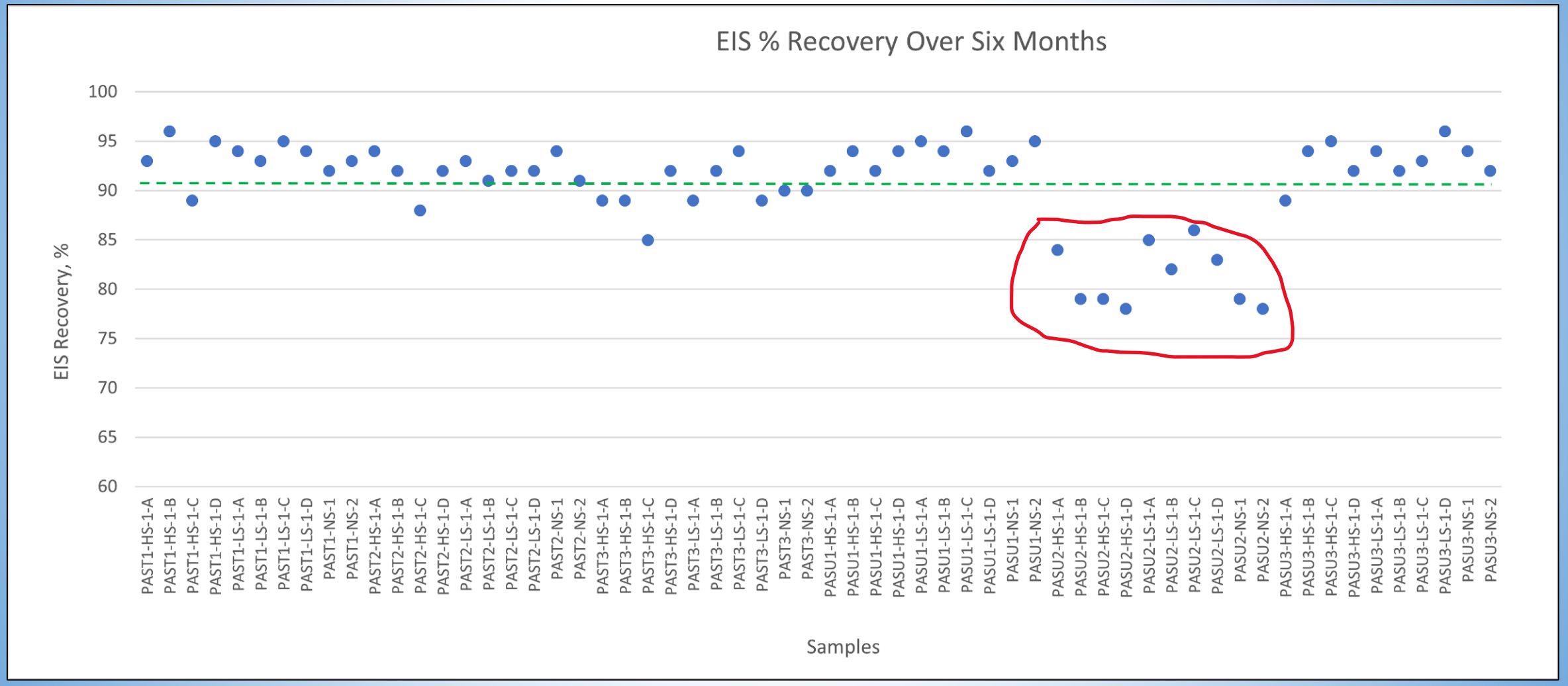
Precision and Accuracy in Surface Water



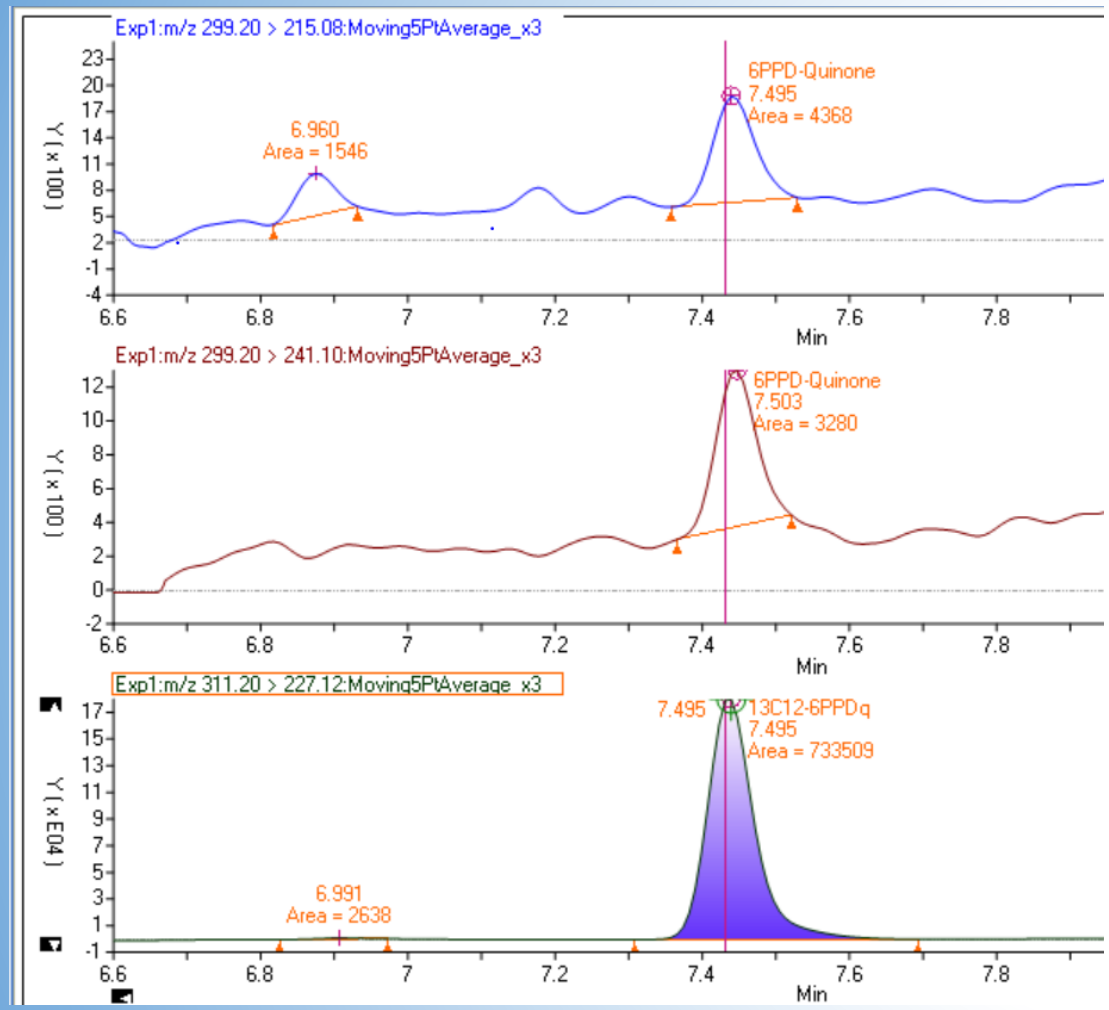
HS = High spike = 200 ppt

LS = Low spike = 20 ppt

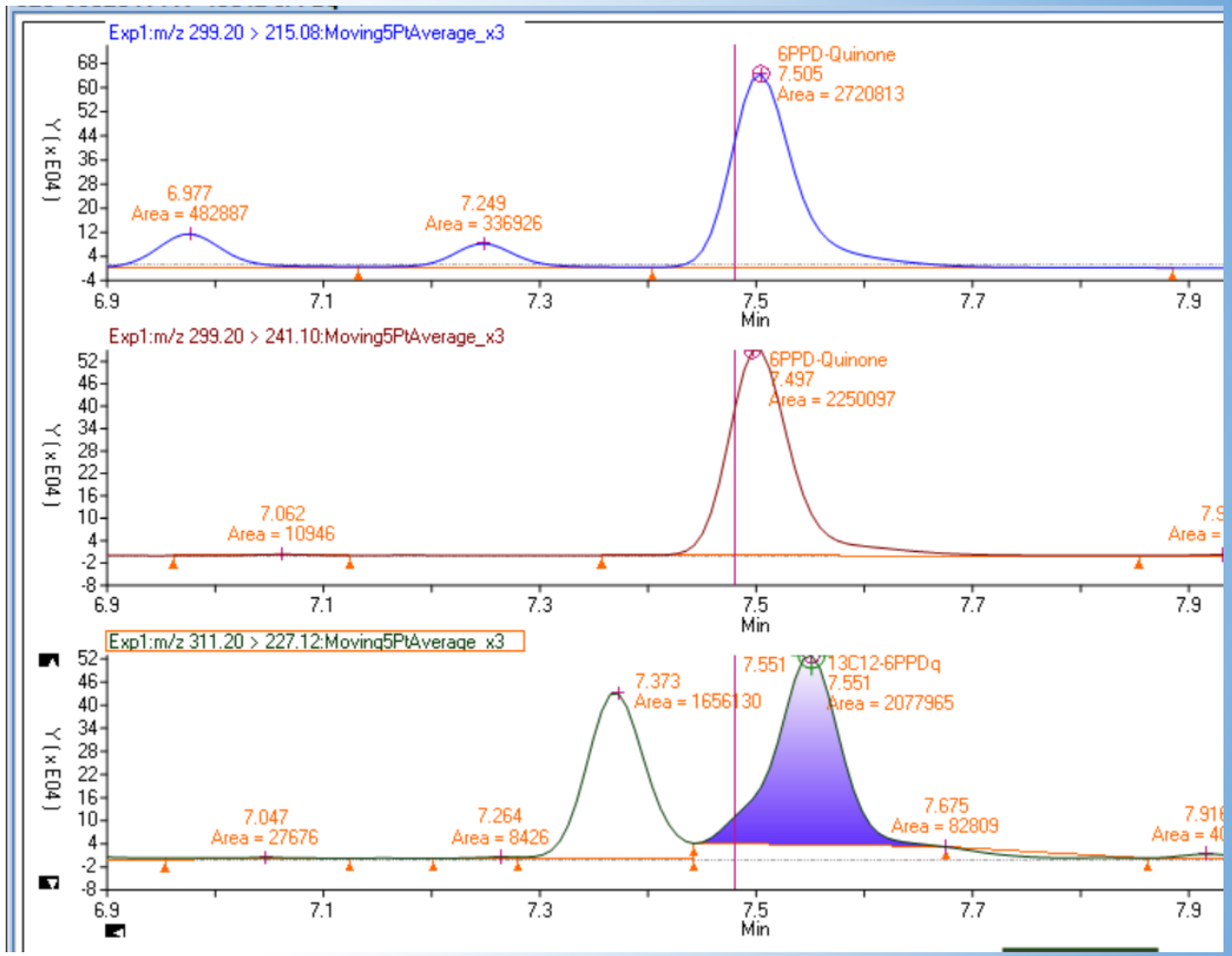
Real-world Sample EIS Results (n=60)



Interference when using $^{13}C_{12}$-6PPD-Q as EIS



EIS normally elutes very close to target analyte



EIS shifted ~0.05 min to the right in some samples

Interference (continued)

- The RT shift for the EIS $^{13}C_{12}$-6PPD-Q quantitation ion was sample-dependent and first seen in some stormwaters
- There were no shifts for the NIS $^{13}C_{6}$-6PPD-Q quantitation ion
- QToF analysis was conducted on the samples containing the interferant. No library match was found, but the interferent differed from the EIS by only 0.0355 amu
- In response, the method was revised to use $^{13}C_{6}$-6PPD-Q as the EIS and use D_{5}-6PPD-Q as the NIS
- CIL was notified of the interference in $^{13}C_{12}$-6PPD-Q and the interference is discussed in Section 4.2 to the draft method

Next Steps

- Analyses of real-world wastewater samples are underway, although this may not be as important a matrix type as stormwater or surface water
- Revising Draft Method 1634 to add wastewater results
- Conducting a multi-laboratory validation study of Draft Method 1634 *if and when funding becomes available*
  - At least 9 labs, such that 6 or more complete the study
  - Real-world samples of stormwaters, surface waters, and wastewaters
  - Study would be designed to support proposal and promulgation of the method at 40 CFR Part 136
  - Data will be used to develop QC acceptance criteria

Other Efforts

- OW recently proposed the following acute aquatic life screening values for 6PPD-Q and 6PPD in water that are protective of sensitive salmon and other aquatic life

6PPD-Q	6PPD	Duration	Frequency
11 ng/L	8,900 ng/L	1 hour	Not to be exceeded more than once in three years, on average

- The screening value for 6PPD-Q is well above the Method 1634 MDL of 0.43 ng/L and the ML of 2.0
- 6PPD itself is very reactive in water, as well as in standards, making quantitative measurements impractical at this time

Other Efforts (continued)

- Region 10 Lab has mobilized the method and applied it to an ORD project in “urban” watersheds. Working it into their accreditation program.
- Region 10 Lab will likely extend the method to soils and tissues next (as time permits)
- Region 10 is in the process of including Method 1634 in an NPDES permit for stormwater monitoring

For More Information See

https://www.epa.gov/cwa-methods/6ppd-q-using-liquid-chromatography-tandem-mass-spectroscopy-lcmsms-method-1634-not-yet

For copies of:

- Draft Method 1634
- The single-lab validation study report

For other news about 6PPD-Q:

https://www.epa.gov/newsreleases/epa-announces-12m-protect-salmon-reducing-toxic-tire-dust-other-pollutants-stormwater

Questions?