



Draft Method 1634: Determination of 6PPD-Quinone in Aqueous Matrices Using Solid Phase Extraction & Liquid Chromatography with Tandem Mass Spectrometry (LC/MS/MS)

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SUMMARY

The degradation of tires on roadways releases various chemicals into the environment, posing ecological and health risks. 6PPD is a compound initially utilized as a rubber antioxidant to increase the longevity of tires. However, it forms 6PPD-quinone (6PPD-Q) when reacting with ozone as tires degrade. This compound has been linked to the mortality of coho salmon exposed to contaminated stormwater, prompting the EPA to release Draft Method 1634 in January 2024 for extracting and analyzing 6PPD-Q in aqueous matrices.

This poster outlines a robust SPE method comparing UCT's Enviro-Clean® high-crosslinked divinylbenzene SPE cartridges (ECHLD156-P) and UCT's Enviro-Clean® hydrophilic-lipophilic-balance (EHLB126-P) SPE cartridges. Recoveries for 6PPD-Q were calculated over a 3-day method detection limit study and mid-level demonstration of capability studies following Draft Method 1634. Studies were also done on tap water to simulate real-world sample extraction. Using UCT's SelectraCore® C18 HPLC Column (SCS27-C18521), an optimized analysis method was developed via LC-MS/MS.

References:
[1] DRAFT Method 1634 Determination of 6PPD-Quinone in Aqueous Matrices Using Liquid Chromatography with Tandem Mass Spectrometry (LC/MS/MS) [USEPA, December 2023] https://www.epa.gov/system/files/documents/2024-01/draft-method-1634-for-web-posting-1-23-24_508.pdf
[2] Appendix B to Part 136, Title 40 of the C.F.R., Definition and Procedure for the Determination of the Method Detection Limit—Revision 2 <https://www.ecfr.gov/current/title-40/chapter-I/subchapter-D/part-136/appendix-Appendix%20B%20to%20Part%20136>
[3] MDL Calculator; Multiple Analyte, Single Template, NCDEQ <https://www.deq.nc.gov/mdl-calculator-multiple-analyte-template/download?attachment>
[4] Treadgold, James William, and James William Treadgold. "The Sources and Environmental Fate of Pharmaceuticals and Personal Care Products in Lowland River Catchments." 2012, <https://doi.org/10.25560/9500>.

INSTRUMENT PARAMETERS

HPLC System	SCIEX Exion LC	
Delay Column	UCT Selectra® C18, 50 × 4.6 mm, 5 µm (p/n: SLC-1850ID46-5UM)	
HPLC Column	UCT SelectraCore® C18, 50 × 2.1 mm, 2.7 µm (p/n: SCS27-C18521)	
Guard Column	UCT SelectraCore® C18, 5 × 2.1 mm, 2.7 µm (p/n: SCS27-C18GDC21) Holder: SLGRDHLDLDR-HPOPT	
Oven Temperature	45°C	
Flow Rate	0.600 µL/min	
Injection Volume	10 µL	
HPLC Gradient		
Time (min)	Mobile Phase A: 2% Formic Acid in Reagent Water	Mobile Phase B: Acetonitrile
0 - 0.4	85	15
1.2	45	55
2.3 - 3.3	1	99
3.4 - 5	85	15
MS/MS System	ABSCIEX Qtrap 6500+	
Ionization Mode	ESI +	
Curtain Gas (CUR)	40	
Collision Gas (CAD)	Medium	
IonSpray Voltage (IS)	5500	
Temperature (TEM)	600	
Ion Source Gas 1 (GS1)	50	
Ion Source Gas 2 (GS2)	50	

CHROMATOGRAMS

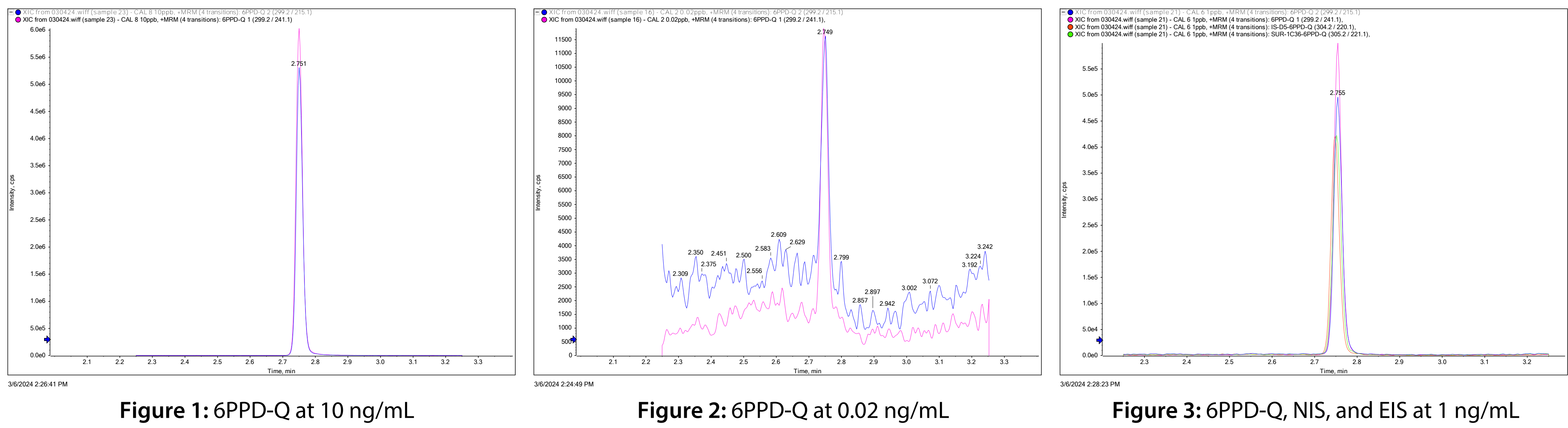


Figure 1: 6PPD-Q at 10 ng/mL

Figure 2: 6PPD-Q at 0.02 ng/mL

Figure 3: 6PPD-Q, NIS, and EIS at 1 ng/mL

SPE PROCEDURE

Sample Pretreatment

- Ensure the pH of the sample is at 5.0 ± 0.5. Adjust with acetate buffer salts/acetic acid if necessary.
 - To adjust pH, add 2.15g (half a pack) of SPHACE5001-10 and 520 µL of glacial acetic acid to an approximately 250 mL sample volume. Verify the sample's pH is 5.0 ± 0.5 with pH strips or a pH meter.
- Record the weight of the container and sample. The weight of the empty container is subtracted from this value to determine the final volume.
- Add 500 µL of 20 µg/L 13C6-6PPD-Q (EIS) to all samples.
- Add 500 µL of 20 µg/L native 6PPD-Q to control spikes.

STEP 1: SPE Conditioning

- Rinse the SPE cartridge with 5 mL acetonitrile.
- Rinse the cartridge with 5 mL of reagent water twice.
- Close the stopcock valve and add 2–3 mL of reagent water.
- Attach adapter and reservoir for loading the sample.

STEP 2: SPE Loading

- Invert the sample bottle to mix and pour the sample into the reservoir.
- Adjust the vacuum so the flow rate is approximately 10 mL/min.

STEP 3: Wash Column

- After loading the sample onto the cartridge, rinse the sample bottle with 5 mL of 50:50 methanol/reagent water and pour it onto the column reservoir.
- After the rinse has passed through the cartridge, allow the cartridge to dry under a high vacuum (10-15-inch Hg) for at least 5 minutes.

STEP 4: Elute Analytes

- Add 15 mL polypropylene centrifuge tubes to the SPE manifold.
- Rinse the sample bottles with 5 mL of acetonitrile.
- Transfer the rinsate to the cartridge reservoir onto the cartridges.
- Adjust the vacuum pressure to elute dropwise.
- Once the eluent passes through the cartridge, pull a high vacuum for ~1 minute to collect as much eluate as possible.
- Repeat steps b and c with a second 4 mL aliquot of acetonitrile.

STEP 5: Extract Volume

- Add 500 µL of 20 µg/L D5-6PPD-Q (NIS) to all eluates.
- Bring to a final volume of 10mL with acetonitrile.
- Cap the centrifuge tube and invert it several times, then vortex.
- Transfer an aliquot to a polypropylene vial for LC/MS/MS analysis.



UCT Part #: ECHLD156-P

CALIBRATION TABLE

Compound	Retention Time	Regression	RSE %	Linear Range (ng/mL)
6PPD-Q	2.75	Linear (1/x ²)	5.2	0.01 - 10.0
¹³ C6-6PPD-Q	2.75	N/A (EIS)	N/A (EIS)	1
D5-6PPD-Q	2.75	N/A (EIS)	N/A (EIS)	1

CALIBRATION CURVES

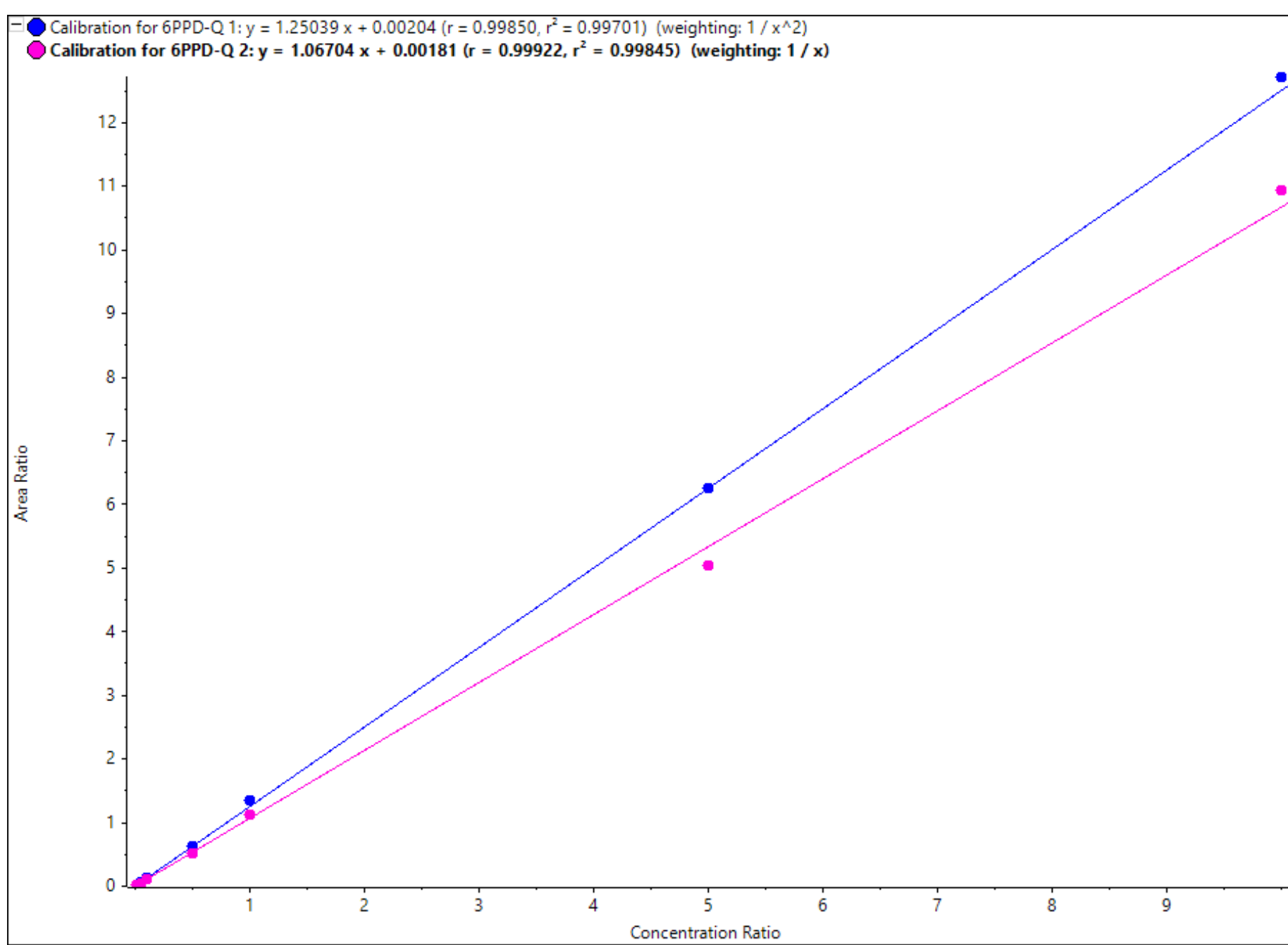


Figure 4: Calibration curve from 0.01 ng/mL to 10 ng/mL

RESULTS

6PPD-Q - (ECHLD156-P) ENVIRO-CLEAN® HLD 500 mg, 6 mL cartridge, PE frits

Results In Reagent Water (n = 4)			Results In Tap Water (acetate buffered, pH 5) (n = 3) *			Results in Reagent water (3 Day MDL Study, n=7)			
Conc (ng/mL)	Recovery %	RSD %	Conc (ng/mL)	Recovery %	RSD %	Conc (ng/mL)	Recovery %	RSD %	On Column MDL (ng/mL)
1.0	106%	1.3	1.0	111%	1.0	0.02	109%	0.22	0.0081

6PPD-Q - (EHLB126-P) ENVIRO-CLEAN® HLB 200 mg, 6 mL cartridge, PE frits

Results In Reagent Water (n = 4)			Results In Tap Water (acetate buffered, pH 5) (n = 3) *			Results in Reagent water (3 Day MDL Study, n=7)			
Conc (ng/mL)	Recovery %	RSD %	Conc (ng/mL)	Recovery %	RSD %	Conc (ng/mL)	Recovery %	RSD %	On Column MDL (ng/mL)
1.0	108%	1.1	1.0	110%	2.2	0.02	110%	0.21	0.0088

* Tap water extracted unbuffered (pH 7) showed poor recovery. Adjusting to pH 5 yielded equivalent recoveries to reagent water.

CONCLUSION

The results demonstrate that 6PPD-Q is extractable using SPE following EPA Draft Method 1634 with UCT's polymeric sorbents ECHLD and EHLB. Recoveries from both sorbents were excellent at low and high concentrations (106-110%). ECHLD achieved a lower method detection limit due to cleaner blanks. It was discovered that adjusting sample pH to 5 greatly improved extraction, leading to comparable recoveries in tap water and reagent water. UCT's SelectraCore® C18 HPLC Column with core-shell technology achieved reduced backpressure and decreased both the run time and injection volume from that described in the draft method. The calibration was improved upon from the draft method to maintain linearity from a range of 0.01-10.0 ng/mL.