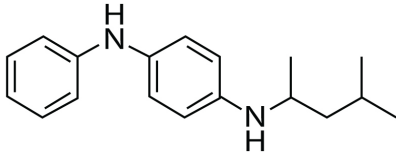
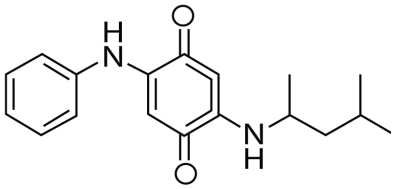
Draft Method 1634: Determination of 6PPD-Quinone in Aqueous Matrices Using Solid Phase Extraction & Liquid Chromatography with Tandem Mass Spectrometry (LC/MS/MS)

6PPD



6PPD-quinone



UCT Part Numbers

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

Or

ECHLB126-P
ENVIRO-CLEAN® HLB 200 mg, 6 mL cartridge, PE frits

SPHACE5001-10
Select pH Buffer Acetate 5.00 pH/100 mM

VMF016GL
Complete 16-Position Glass Block Manifold

RFV00150P
Empty Polypropylene Reservoirs

AD0000AS
Cartridge Adapter

SCS27-C18521
SelectraCore® C18 HPLC Column (50 × 2.1 mm, 2.7 µm)

SCS27-C18GDC21
SelectraCore® C18 Guard Column (5 × 2.1 mm, 2.7 µm)

SLGRDHLDR-HPOPT
Selectra® Direct Connect Guard Holder

Summary:

The degradation of tires on roadways is known to release numerous chemicals into the environment, posing ecological and health risks. To combat this, the presence of 6PPD (N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine) gained attention due to its role as a rubber antioxidant. However, studies now show that through reactions with environmental oxidants such as ozone, this compound forms 6PPD-quinone (6PPD-Q), a highly reactive and toxic compound that can pose risks to ecosystems and human health. It has popularly been linked to the mortality of coho salmon as they migrate upstream to spawn, so much so that only a few hours of exposure of the salmon to contaminated stormwater has proven lethal. Considering these recent studies, the EPA released an official Draft Method 1634 in January 2024 to extract and analyze 6PPD-Q in water. This method uses an extracted internal standard (EIS) to measure the concentration of 6PPD-Q by isotope dilution and a native internal standard (NIS) to measure the efficiency of the extraction procedure.

This application outlines the SPE extraction of water samples following draft method 1634, comparing both UCT's Enviro-Clean® polymeric high-crosslinked divinylbenzene SPE cartridges (**ECHLD156-P**) and UCT's Enviro-Clean® polymeric hydrophilic-lipophilic-balance (**ECHLB126-P**) SPE cartridges. Both cartridges demonstrated efficient recoveries over a 3-day method detection limit study and mid-level demonstration of capability studies. ECHLD156-P demonstrated the best MDL values and cleanest blanks. Using UCT's SelectraCore® C18 HPLC Column (**SCS27-C18521**), an optimized analysis method was developed using LC/MS/MS. The LC method was shortened to a 5-minute run time and 10 microliter injection volume was used.

The MS/MS method reached sensitivity levels below the EPA's lowest suggested standard and met all method criteria for linearity and reproducibility.

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Sample Pretreatment:

1. Ensure the pH of the sample is at 5.0 ± 0.5. Adjust with acetate buffer salts/acetic acid if necessary.
    a) 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.
2. Record the weight of the container and sample. The weight of the empty container is subtracted from this value to determine the final volume.
3. Add 500 µL of 20 µg/L 13C6-6PPD-Q (EIS) to all samples.
4. Add 500 µL of 20 µg/L native 6PPD-Q to control spikes.

SPE Procedure:

The cartridge should not go dry until after the final rinse. Stop the drip of the solvent/sample when it reaches the top frit.

1. SPE Conditioning

a) Rinse the SPE cartridge (**ECHLD156-P** OR **ECHLB126-P**) with 5 mL acetonitrile.
b) Rinse the cartridge with 5 mL of reagent water twice.
c) Close the stopcock valve and add 2–3 mL of reagent water.
d) Attach adapter AD0000AS and reservoir **RFV00150P** for loading the sample.

2. SPE Loading

a) Invert the sample bottle to mix and pour the sample into the reservoir.
b) Adjust the vacuum so the flow rate is approximately 10 mL/min.
b1) If the SPE column should plug (flow rate <1 drop per minute) before all of the sample passes through the cartridge, do the following:
    1. Return any remaining sample volume to the original container.
    2. Weigh the container and record this weight.
    3. Determine the sample volume extracted by subtracting the remaining sample weight from the initial weight.
    4. Continue to Step 3.

3. Wash Column

a) 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. (If the cartridge had plugged and the steps in section 2-b1 were performed, apply the wash directly to the reservoir. Any potential loss of analyte that might adhere to the walls of the bottle is a minor concern.)
b) 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.

4. Elute Analytes

a) Add 15 mL polypropylene centrifuge tubes to the SPE manifold.

b) Rinse the sample bottles with 5 mL of acetonitrile. (If the cartridge had plugged and the steps in section 2-b1 were performed, apply the rinse directly to the reservoir. Any potential loss of analyte that might adhere to the walls of the bottle is a minor concern.)

c) Transfer the rinsate to the cartridge reservoir onto the cartridges.

d) Adjust the vacuum pressure to elute dropwise. (If the lab uses a siphon or automated SPE system, the rinse should include the original sample bottle and the flow path.)

e) Once the eluent passes through the cartridge, pull a high vacuum for ~1 minute to collect as much eluate as possible.

f) Repeat steps **b** and **c** with a second 4 mL aliquot of acetonitrile. The total volume collected should be approximately 9-10 mL.

5. Final Extract Volume

a) Add 500 µL of 20 µg/L D5-6PPD-Q (NIS) to all eluates.

b) Bring to a final volume of 10mL with acetonitrile. (If an extract requires reanalysis and evaporation has occurred, do not add additional NIS. Return the extract to its previous volume with acetonitrile).

c) Cap the centrifuge tube and invert it several times, then vortex.

d) Transfer an aliquot to a polypropylene vial for LC/MS/MS analysis.

HPLC Parameters:

HPLC Conditions	
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: **SLGRDHLDR-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 (aq)	Mobile Phase B (%) Acetonitrile
0	85	15
0.4	85	15
1.2	45	55
2.3	1	99
3.3	1	99
3.4	85	15
5	85	15

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MS/MS Parameters:

MS Conditions	
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

MRM Table					
Compound	**Transition**	**DP (V)**	**EP (V)**	**CE (V)**	**CXP (V)**
6PPD-Q	299.2 > 241.1	100	10	38	9
6PPD-Q	299.2 > 215.1	22	10	25	9
13C6-6PPD-Q	305.2 > 221.1	106	10	25	9
D5-6PPD-Q	304.2 > 220.1	106	10	23	9
* 299.2 > 241.1 showed the most stability and was chosen as the quantifier for this analysis.					

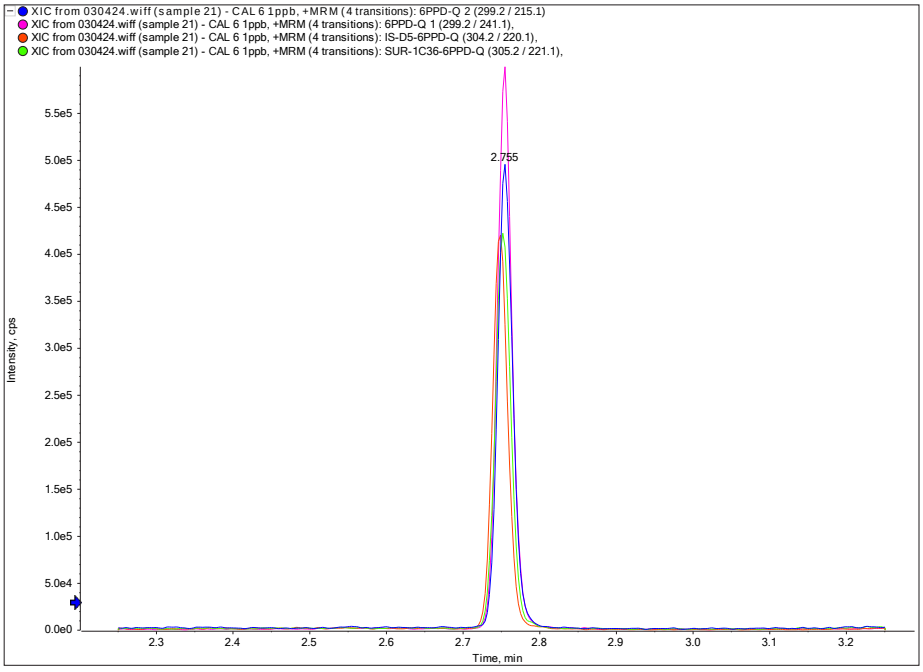

Figure 1: Analytes and Internal Standards at 1 ng/mL

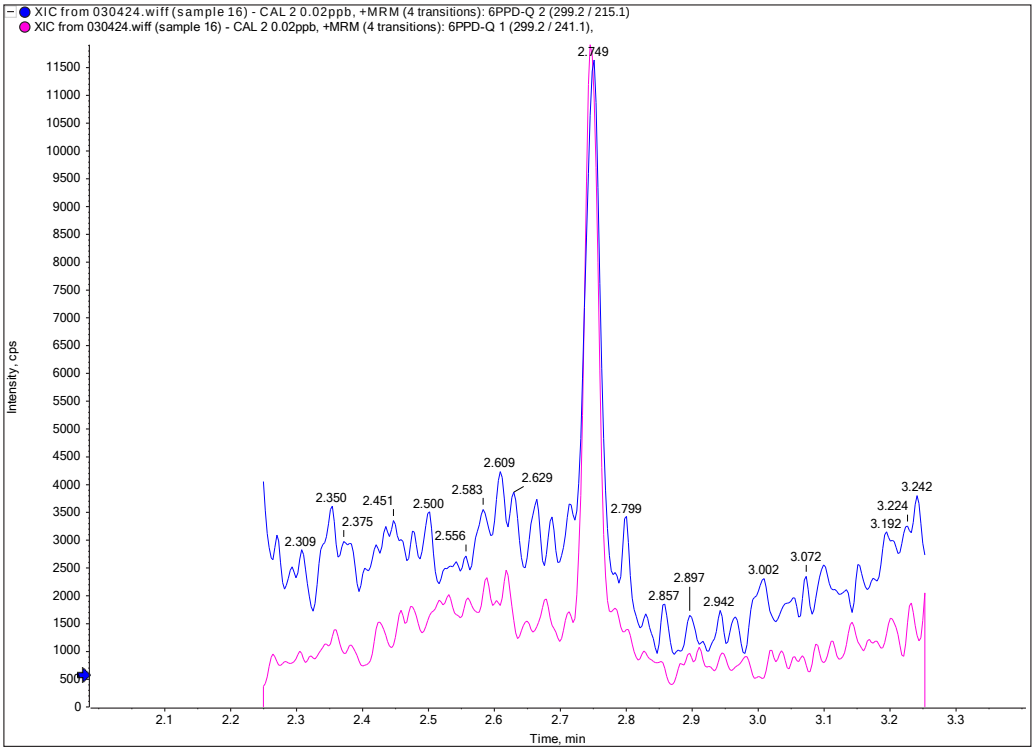

Figure 2: 6PPD-Q at 0.02 ng/mL

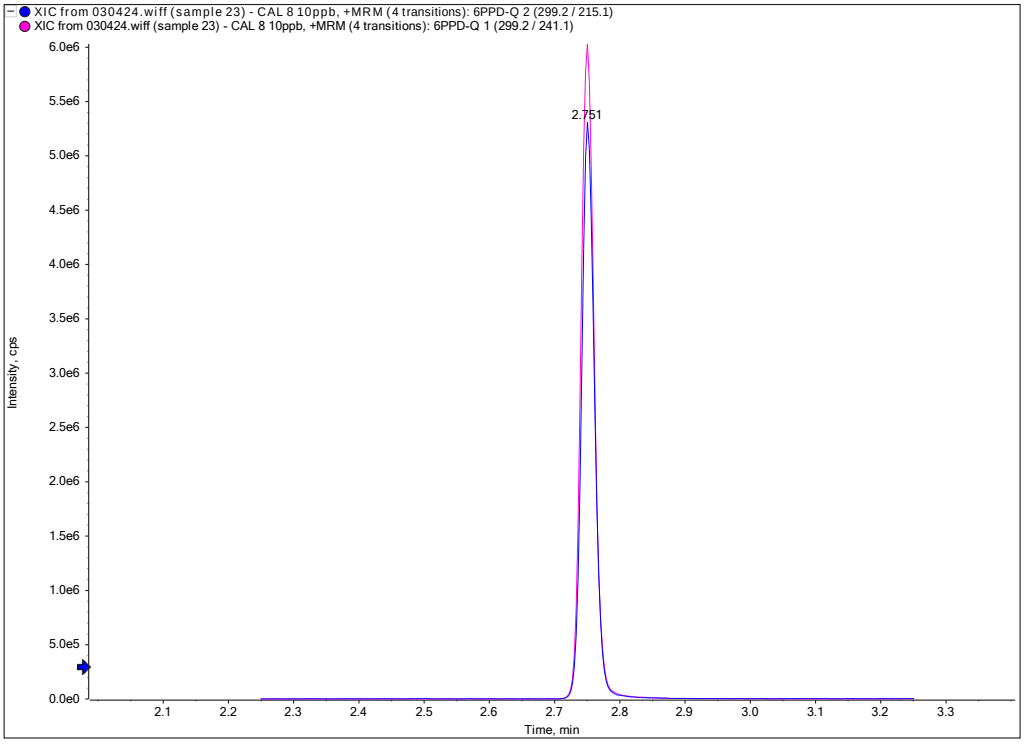

Figure 3: 6PPD-Q at 10 ng/mL

Calibration Curve:

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 (NIS)	N/A (NIS)	1

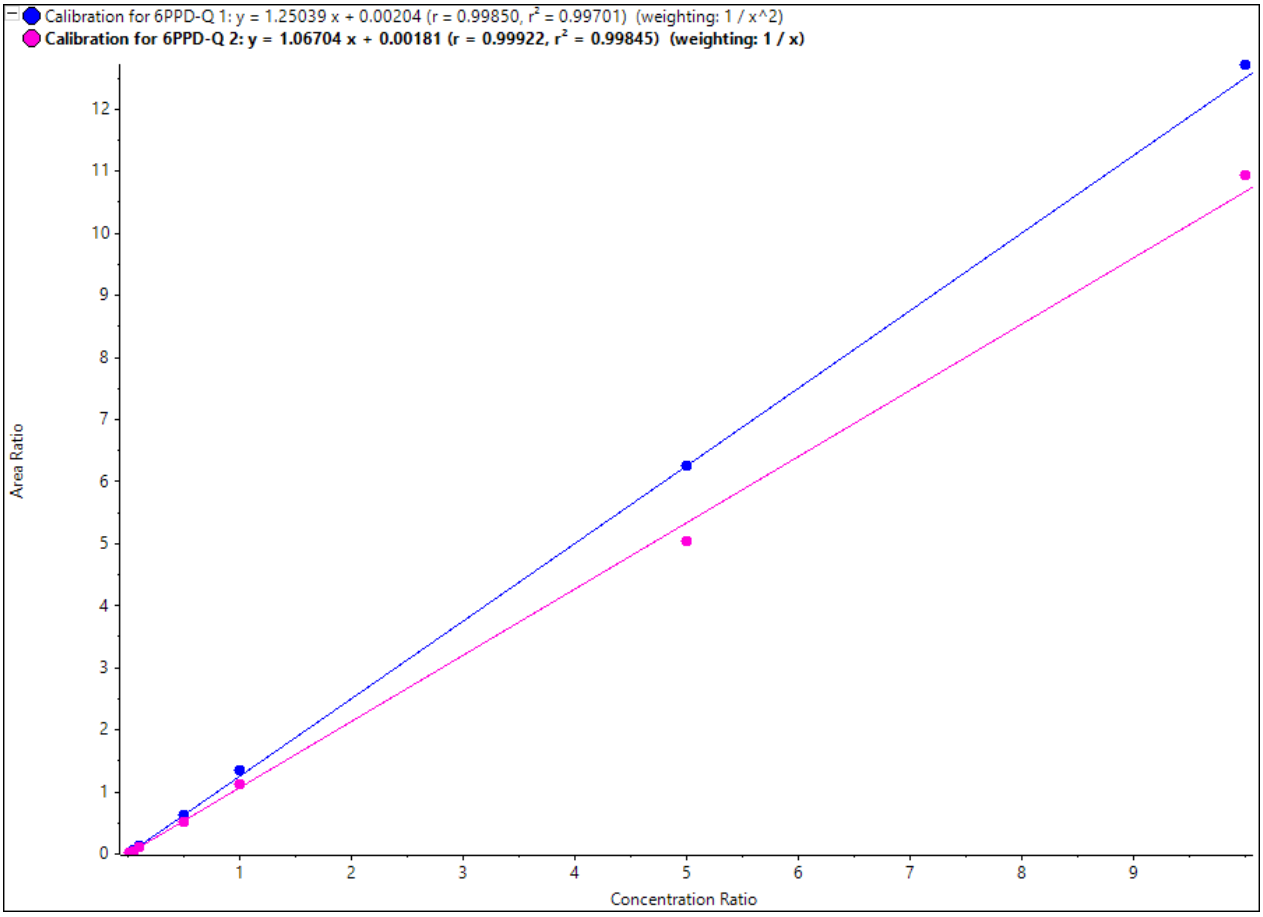

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Figure 4: Calibration for 6PPD-Q from 0.01 - 10 ng/mL

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 - (ECHLB126-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/Discussion:

These results demonstrate that 6PPD-Q is extractable by SPE using two different UCT polymeric sorbents, highly crosslinked divinylbenzene (ECHLD) and hydrophilic-lipophilic-balanced (ECHLB).

The MDL for 6PPD-Q was prepared and calculated using the MDL procedure in 40 CFR Part 136, Appendix B (Reference 10). An Initial Demonstration of Capability was performed using both sorbents according to procedures in section 9.2 of Draft Method EPA 1634. While both sorbents showed excellent recovery (between 106 % – 110 %) at both high (n=4) and low (n=7) levels in reagent water, **ECHLD produced cleaner blanks and achieved a lower MDL.**

Sample pH is critical for the proper adsorption of the analytes to sorbents. Poor recoveries occurred during a preliminary extraction of tap water with both sorbents using the EIS 13C6-6PPD-Q and the target analyte 6PPD-Q. When tap water samples were adjusted to pH 5 using the acetate buffer SPHACE5001-10, recoveries were equal to the extractions in reagent water in both sorbents.

UCT's SelectraCore® C18 HPLC Column contains core-shell technology, which reduces backpressure on the LC and band broadening in the chromatography. The run time and injection volume achieved with this column halves the time and volume used in the draft method, allowing for faster analyses and less solvent usage. The calibration resolved 6PPD-Q down to 0.01 ng/mL with acceptable signal-to-noise levels, allowing for a more comprehensive calibration range than currently proposed in the draft method while maintaining linearity. All ongoing QC requirements in sections 9.0 and 13.0 of draft method 1634 were achieved.

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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.

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