

# Quantitation of Toxic Tire Degradant 6PPD-Quinone in Surface Water

Using direct injection on an Agilent 6470 triple  
quadrupole LC/MS



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## Abstract

The widely used tire-rubber stabilizer 6PPD (N-1,3-dimethylbutyl-N'-phenyl-*p*-phenylenediamine) degrades to form 6PPD-quinone, which is toxic to juvenile coho salmon and potentially toxic to other aquatic species. This application note describes a fast, direct-inject analytical method for the quantitation of 6PPD-quinone in surface water using liquid chromatography/triple quadrupole mass spectrometry (LC/TQ). Sample preparation, recovery, precision, stability, and reporting limit is described.

## Introduction

Recent work by Tian *et al.*<sup>1</sup> demonstrated that the commonly used tire antioxidant 6PPD degrades to form a transformation product known as 6PPD-quinone. This degradant was shown to be highly toxic to juvenile coho salmon and proposed to be toxic to other aquatic species. 6PPD-quinone is detectable in roadway runoff and waterways affected by urban runoff. Tian *et al.* reported a median lethal concentration ( $LC_{50}$ ) of  $0.8 \pm 0.16$   $\mu\text{g/L}$ .

This application note establishes a fast, easy, triple quadrupole LC/MS method for 6PPD-quinone. The analysis uses direct injection of surface water samples with the addition of internal standard  $D_5$ -6PPD-quinone and a reporting limit of  $0.02$   $\mu\text{g/L}$ .

Numerous columns and mobile phase combinations were evaluated. The best response was achieved on a  $2.1 \times 50$  mm,  $1.9$   $\mu\text{m}$  Agilent InfinityLab Poroshell 120 EC-C18 column with mobile phases of ammonium fluoride and acetonitrile.

The parent compound 6PPD was evaluated but not presented in this method due to the rapid degradation of 6PPD in water.<sup>2</sup> 6PPD also gives poor chromatographic performance due to the presence of multiple charge states in solution. For 6PPD evaluation, care must be taken to control and maintain the solution pH, and compound breakdown should be factored in.

Method validation was evaluated including precision, accuracy, lowest concentration minimum reporting level (LCMRL), and stability. A selection of real-world samples were collected and analyzed for demonstration of proof of concept.

## Experimental

6PPD-quinone analysis was performed using an Agilent 1290 Infinity II LC coupled to an Agilent 6470 LC/TQ. LC conditions are provided in Table 1. The system was controlled by Agilent MassHunter Acquisition software version 10.1. Data processing was performed with MassHunter Quantitative Analysis software version 10.1 and MassHunter Qualitative Analysis software version 10.0. LC/TQ method parameters are listed in Table 2.

## LC instrument conditions

Table 1. LC method parameters for 6PPD-quinone.

Parameter	Value										
LC	Agilent 1290 Infinity II LC: Agilent 1290 Infinity II multisampler (G7167B), Agilent 1290 Infinity II high-speed pump (G7120A), and Agilent 1290 Infinity II multicolumn thermostat (G7116B)										
Analytical Column	Agilent InfinityLab Poroshell 120 EC-C18, $2.1 \times 50$ mm, $1.9$ $\mu\text{m}$ (p/n 699675-902)										
Column Temperature	$40$ $^{\circ}\text{C}$										
Injection Volume	$5$ $\mu\text{L}$										
Mobile Phase	A) $1$ mM ammonium fluoride in water (HPLC grade) B) acetonitrile (HPLC grade)										
Flow Rate	$0.6$ mL/min										
Gradient	<table border="1"><thead><tr><th>Time (min)</th><th>% B</th></tr></thead><tbody><tr><td>0.0</td><td>30</td></tr><tr><td>0.5</td><td>30</td></tr><tr><td>4.7</td><td>70</td></tr><tr><td>4.8</td><td>100</td></tr></tbody></table>	Time (min)	% B	0.0	30	0.5	30	4.7	70	4.8	100
Time (min)	% B										
0.0	30										
0.5	30										
4.7	70										
4.8	100										
Stop Time	$5.7$ min										
Post Time	$1.5$ min										

## LC/TQ instrument conditions

Table 2. LC/TQ method parameters for 6PPD-quinone.

Parameter	Value
MS	Agilent 6470 triple quadrupole LC/MS with Agilent Jet Stream ESI source (G6470B)
Source Parameters	
Gas Temperature	$300$ $^{\circ}\text{C}$
Gas Flow	$10$ L/min
Nebulizer	$40$ psi
Sheath Gas Temperature	$375$ $^{\circ}\text{C}$
Sheath Gas Flow	$11$ L/min
Capillary Voltage (Neg)	$2,500$ V
Nozzle Voltage (Neg)	$0$ V
Delta EMV (+)	$400$

## MRM transitions

The method used multiple reaction monitoring (MRM) and was run in electrospray positive mode using a 6470 LC/TQ. All compound parameters including precursor ion, product ion, fragmentor voltages, and collision energies were optimized with MassHunter Optimizer software. MRM transitions are shown in Table 3.

**Table 3.** MRM transitions for 6PPD-quinone and ISTD.

Compound Name	Precursor Ion (m/z)	Product Ion (m/z)	Ret. Time (min)	Fragmentor (V)	Collision Energy (V)	Cell Accelerator Voltage	Polarity
6PPD-Quinone	299.2	241.1	3.52	105	32	4	Positive
6PPD-Quinone	299.2	215.1	3.52	105	16	5	Positive
6PPD-Quinone	299.2	187.1	3.52	105	32	5	Positive
D <sub>5</sub> -6PPD-Quinone	304.2	246.1	3.49	110	36	4	Positive
D <sub>5</sub> -6PPD-Quinone	304.2	220.1	3.49	110	20	4	Positive
D <sub>5</sub> -6PPD-Quinone	304.2	192.1	3.49	110	36	5	Positive

## Preparation of calibration standards

6PPD-quinone and internal standard D<sub>5</sub>-6PPD-quinone stock standards (both at 100 µg/mL) were purchased from HPC Standards Inc. A high calibrator (50 ng/mL) was prepared in HPLC grade water by dilution of a 1,000 ng/mL working standard in acetonitrile. Lower calibrators were prepared by 1:4 serial dilutions of the high calibrator in water. Internal standard spiking solution was prepared at 500 ng/mL in acetonitrile. Prepared calibrators were spiked with internal standard resulting in a concentration of 5 ng/mL in each vial.

## Sample preparation

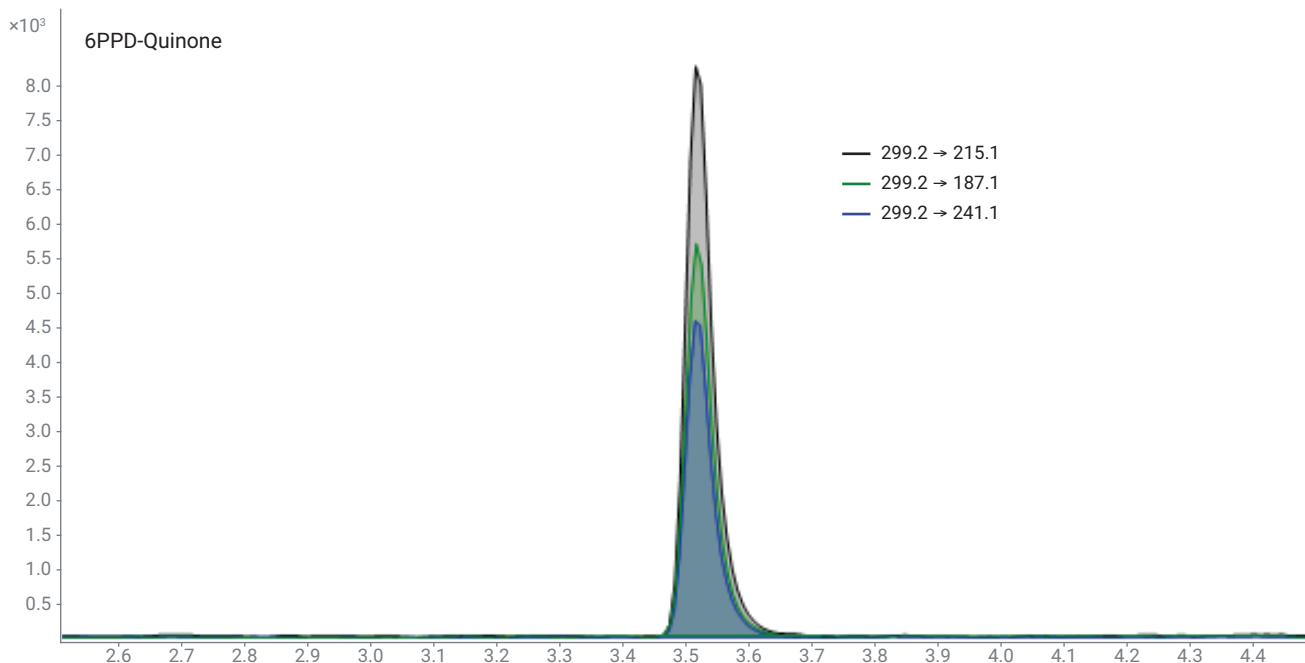
Surface water (for a negative control) was sampled from a local stream during a time without road runoff. Potential positive samples were collected from residential roadside drainage during a rainstorm and a residual puddle in a parking lot. These samples were stored at 4 °C before analysis.

- Precision and accuracy samples were prepared in HPLC grade water and stream water by spiking five replicate 1-mL samples at 5 ng/mL. LCMRL replicate spikes (n = 4) were prepared in HPLC grade water at seven concentrations ranging from 0.02 to 3 ng/mL.
- All samples were centrifuged for 5 minutes at 13,000 rpm, then a 1-mL aliquot was transferred directly into an autosampler vial.
- All samples were spiked with internal standard to a resulting 5 ng/mL concentration in the vial before LC/TQ analysis.

## Results and discussion

### Chromatography

Figure 1 shows the chromatography for 6PPD-quinone at 0.2 ng/mL (25% of the median lethal concentration). The peak height and signal-to-noise clearly show that quantification can be done at much lower levels, highlighting the enhanced sensitivity of the 6470 LC/TQ.



**Figure 1.** 6PPD-quinone chromatography at 0.2 ng/mL.

### Method performance

6PPD-quinone was quantitated using a calibration curve from 0.01 to 50 ng/mL using quadratic fit, 1/x weighting, and including the origin. The  $R^2$  value was greater than 0.999.

Method recovery (accuracy) in HPLC water was 113.5% and in stream water was 112.6%. Precision, expressed as %RSD, was 3% in HPLC water and 1% in stream water for  $n = 5$  replicates at 5 ng/mL. The LCMRL of 6PPD-quinone was 0.023 ng/mL. There was no contribution to the 6PPD-quinone signal from the LC system.

To demonstrate stability, a single midlevel calibrator (3.125 ng/mL) was injected repeatedly over the course of three days (67 hours) uninterrupted & interspersed with 172 sample & QC injections and slow-flow mobile phase conservation methods. Figure 2 shows the overlay of 17 injections over three days showing the RT and abundance stability across the batch.

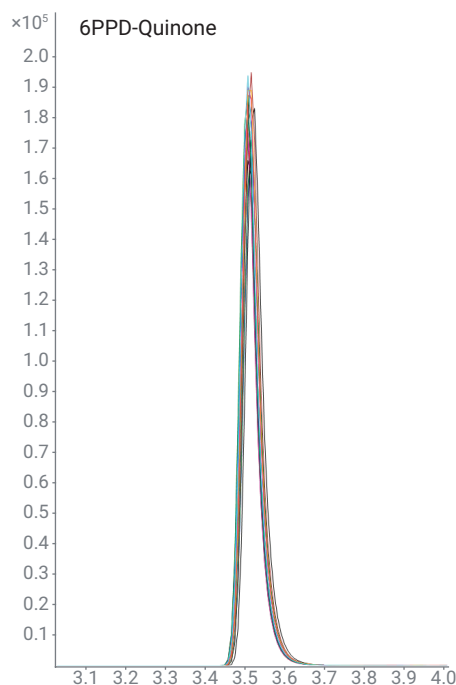


Figure 2. 6PPD-quinone stability over three days.

The real-world samples that were collected and analyzed tested positive for 6PPD-quinone. The runoff from a residential street during a rain event had 0.06 ng/mL and the parking lot puddle had 0.27 ng/mL of 6PPD-quinone. These samples demonstrate the applicability of this method to a variety of field samples.

## Conclusion

This application note has demonstrated a fast method (under eight minutes injection to injection) for the quantitative analysis of the toxic tire degradant 6PPD-quinone in surface water using an Agilent 6470 LC/TQ. The methodology involves minimal sample preparation (centrifugation), isotopic internal standard dilution, and direct injection. This work exhibited a low LCMRL of 0.023 ng/mL, nearly 40-times lower than the reported median lethal concentration of 0.8 µg/L. Method recovery was demonstrated in both HPLC water and surface water as 113.5 and 112.6% respectively with precision of 3 and 1%. Instrument stability was demonstrated over a three-day worklist. Finally, samples of roadside rain runoff and a residual parking lot puddle were tested to demonstrate method applicability, and both tested positive for 6PPD-quinone.

## References

1. Tian, *et al.* A Ubiquitous Tire Rubber–Derived Chemical Induces Acute Mortality in Coho Salmon. *Science* **2021**, 371(6525), 185–189.
2. Results of the Evaluation of the PBT/VPVB Properties of: Substance Name: N-1,3-Dimethylbutyl-N'-phenyl-p-phenylenediamine (6PPD). ECB – Summary Fact Sheet, PBT Working Group – PBL List No.97.

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