Analysis of 6-PPD-Quinone in environmental waters

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Introduction

Attention was recently brought to N-(1,3-dimethylbutyl)-N′-phenyl-p-phenylenediamine-quinone (6-PPD-quinone) because of its role in the mortality of Coho salmon population in the Pacific Norwest streams. 6-PPD-quinone is an oxidation product of N-(1,3-dimethylbutyl)-N′-phenyl-p-phenylenediamine (6PPD). 6-PPD is used ubiquitously in tire rubber as an antioxidant. 6-PPD when released from vehicle tires gets converted to 6-PPD-quinone by reaction with ozone; the chemicals find their way to the streams where Coho salmon inhabits through stormwater run-off. Occurrence of 6-PPD-quinone is linked to a major environmental emerging concern, the ubiquitous distribution of tire-ware particles in the environment.

In this work we evaluated the performance of LCMS-8060 triple quadrupole mass spectrometer (Figure 1) for the analysis of 6-PPD-quinone in environmental waters.

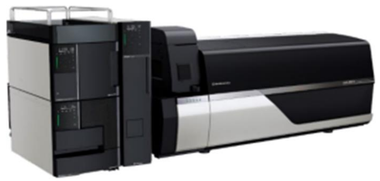

Figure 1. Nexera HPLC with LCMS-8060

Experimental Approach

LCMS grade solvents were purchased from Honeywell (Charlotte, NC). 6-PPD-quinone standard was purchased from HPC Standards Inc. 6-PPD-quinone (0.9 mg) standard was first dissolved in 0.5 mL toluene and then 0.4 mL acetonitrile was added to obtain a 1 mg/mL solution. MRM optimization was performed using flow injection analysis (FIA) and using the LabSolutions automated algorithm. One quantifier and two qualifier ions were selected for the MRM analysis (Table 1). Optimized LCMS conditions used for the analysis are listed in Table 2.

Subsequent dilutions were prepared in 1:1 acetonitrile water to obtain calibrants in 0.01-100 ng/mL range and injected in triplicate. For spiking experiments, water was collected from Savage river, Columbia, MD and a local stream. The water was spiked with 6-PPD-quinone at 0.1, 1, 10, and 50 ng/mL and was analyzed by direct injection without further sample treatment.

Table 1. MRM transitions used for the analysis of 6-PPD-quinone. The MRM transition highlighted in bold is the quantifier ion

Compound	Polarity	MRM transition (*m/z*)	CE (V)
6-PPD-quinone	Positive	**299.20>241.15** 299.20>187.15 299.20>215.10	-30 -29 -20

Table 2. LCMS parameters

LCMS-8060	Parameters	LC-40	Parameters
Ion source	ESI	**Column**	Phenomenex Kinetex 1.7 µm, C18, 50x2.1 mm
Nebulizing gas	2 L/min	**Flow rate**	0.4 mL/min
Interface temperature	300 °C	**Mobile phase A**	A. 0.1% formic acid in water B. Acetonitrile
DL temperature	250 °C	**Injection volume**	5 µL
Heat block temperature	400 °C	**Autosampler temperature**	15 °C
Drying gas	10 L/min	**Column oven**	40 °C
Polarity	Positive	**Run time**	13 min

Results and Discussion

The MRM optimization was performed on neat 6-PPD-quinone standard. Three MRM transitions, based on the product ions observed in CE select data and shown in Table 1, were selected for chromatographic method development. Figure 2a shows an example MRM chromatogram for the quantifier ion m/z 299.20>241.15 at 1 ng/mL in neat standard.

Figure 1b shows the 6-PPD-quinone calibration curve; excellent linearity was observed for 6-PPD-quinone in the selected 0.01-100 ng/mL range (r2 >0.999, weighting 1/c). The percent accuracy for the individual injections of calibrants ranged from 85.3-122.3%; the average % accuracy of the triplicated injections ranged 91.9-110.6. Results from the individual injections are shown in Table 3. %RSD (2.3-9.6) is also included.

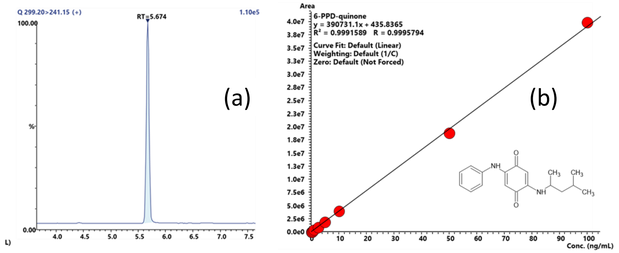

Figure 2. (a) Linear calibration curve for 6-PPD-quinone in the 0.01-100 ng/mL range. Inset: Chemical structure of 6-PPD-quinone. **(b)** Example MRM chromatogram for 6-PPD-quinone (*m/z* 299.20 > 241.15). Concentration of neat standard is 1 ng/mL.

Table 3. Area, %accuracy, and %RSD for all the different calibration levels.

Calibrant (ng/mL)	Average area	% Accuracy	Average % Accuracy	%RSD
0.01	4,619	107.9 101.5 122.3	110.6	9.6
0.05	20,681	106.8 103.4 102.3	104.2	2.3
0.1	39,544	101.4 104.6 94.8	100.3	5.0
0.5	180,222	85.3 97.6 92.9	91.9	6.8
1.0	392,026	100.1 105.5 94.7	100.1	5.4
2.5	935,349	100.4 98.4 87.9	95.6	7.0
5	1,884,125	97.7 90.3 100.7	96.2	5.6
10	4,033,179	95.2 107 106.9	103.0	6.6
50	19,194,400	91.8 101.2 101.3	98.1	5.6
100	39,177,396	96.4 95.5 108.3	100.1	7.1

Water from low urbanized areas (Savage River and a local stream near Columbia, MD) was collected and spiked at 0.1, 1, 10, and 50 ng/mL 6-PPD-quinone. The spike recovery was 100 and 90% at the lowest level spiked (0.1 ng/mL) for local stream and river, respectively. The overall spike recovery at all other concentrations ranged from 83-100% (Table 4) demonstrating excellent sensitivity and low to none matrix-related signal suppression. 6-PPD-quinone was not detected in the stream sample and its concentration was not quantifiable in the river sample; overlay of MRMs from samples analyzed are shown in Figure 5.

Table 4. % recovery of 6-PPD-quinone spiked in samples.

	Local stream	Savage river, MD
Spike level (ng/mL)	**% recovery**	**% recovery**
0.1	100.0	90.0
1	95.7	83.2
10	96.7	93.3
50	96.3	98.4

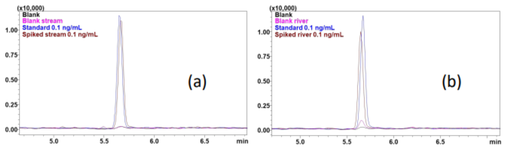

Figure 5. MRM overlay in (a) blank, standard, unspiked and spiked stream samples, (b) blank, standard, unspiked and spiked river samples.

Conclusions

- A rapid and reliable identification and quantitation method for the analysis of 6-PPD-quinone was developed using LCMS-8060 instrument.
- Excellent sensitivity and linearity (r2>0.99) was observed in the 0.01-100 ng/mL range. The overall accuracy for neat standards was in 85-122% range.
- The spike recovery of 6-PPD-quinone in local stream and river water samples was also tested. The method demonstrated excellent %recovery (83-100%) at varying spike levels (0.1, 1, 10, and 50 ng/mL) with no sample clean-up step or use of internal standard.
- LCMS-8060 proved to be an excellent instrument of choice for the selectivity, sensitivity, linearity, and accuracy desired for the analysis of 6-PPD-quinone in environmental waters with minimal sample prep.