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Application News

SSI-LCMS-133

Liquid Chromatography Mass Spectrometry

Analysis of 6-PPD-Quinone in River Water and Local Stream Matrix using LCMS-8060 Triple Quadrupole Mass Spectrometer

Liquid Chromatograph Mass Spectrometer
LCMS-8060NX

■ Summary

LCMS-8060 triple quadrupole mass spectrometer was used for the rapid analysis of N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine-quinone (6-PPD-quinone) in surface water. The performance of a direct injection method was demonstrated by determining the linear range, accuracy, precision and spike recovery. The linear range in neat solution was from 0.01-100 ng/mL (r2 0.9992). The spike recovery of 6-PPD-quinone in two different surface water samples collected from a local stream and river ranged between 83% and 100%, when samples were spiked with concentrations between 0.1 and 50 ng/mL. Concentration of 6-PPD-quinone was not detected in the stream water and below quantifiable concentration in the river water. This work demonstrates the suitability of Shimadzu's LCMS-8060 for the rapid analysis of emerging contaminants, such as 6-PPD-quinone, at environmentally relevant concentrations.

■ Background

Contamination of surface water by tire wear particles is widespread and dramatically impacts ecosystems. An example of the effects from these particles is the acute mortality of Coho salmon in the US Pacific Northwest. Tian et. al.[1] characterized the toxic fractions of stormwater and roadway runoff the Coho salmon were exposed to and ultimately identified N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine-quinone (6-PPD-quinone) as the culprit of the mortality. 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; it eventually leaches from the tire material during its use.

A rapid LC-MS/MS method for the analysis of 6-PPD-quinone by direct injection using the LCMS-8060 triple quadrupole mass spectrometer was developed in this work to aid in the characterization of contamination from tier wear particles.

■ Method

LCMS grade solvents were purchased from Honeywell (Charlotte, NC). The 6-PPD-quinone standard was purchased from HPC Standards Inc. (Atlanta, GA).

LCMS-8060 triple quadrupole mass spectrometer was coupled to a Nexera HPLC system. Table 1a shows the LC and MS parameters used for this analysis and table 1b shows the chromatographic gradient. The MRM optimization was performed for the standards using the MRM optimization tool in LabSolutions (v. 5.99SP2) using flow injection analysis (Table 1c).

6-PPD-quinone (0.9 mg) standard was first dissolved in toluene (0.5 mL) and further diluted with 0.4 mL of acetonitrile to obtain a 1 mg/mL solution. Subsequent dilutions were prepared in 1:1 acetonitrile:water to obtain calibration standards in the 0.01-100 ng/mL range; each standard was injected in triplicates.

For spiking experiments, water was collected from Savage River and a local stream, both in Columbia, MD. The surface water samples were spiked with 6-PPD-quinone at 0.1, 1, 10, and 50 ng/mL, and analyzed as-is without any further sample clean-up.

Table 1a: LC and MS parameters.

LCMS-8060	Parameters	Nexera HPLC	Parameters
Ion Source	ESI	Column	Phenomenex Kinetex 1.7 µm, C18, 50x2.1 mm (Part no. 00B-4475-AN)
Nebulizing Gas	2 L/min	Mobile phase	A: 0.1% formic acid in water B: Acetonitrile
Interface Temperature	300 °C	Flow rate (mL/min)	0.4
DL Temperature	250 °C	Injection volume (µL)	5
Heat Block Temperature	400 °C	Autosampler temperature (°C)	15
Drying Gas	10 L/min	Column Oven (°C)	40
Polarity	Positive	Run time	13 min

Table 1b: Chromatographic gradient used for the analysis.

Time (min)	%B
0	5
1	5
4	50
10	100
11	100
11.1	5
13	Stop

Table 1c: MRM transitions used for the analysis of 6-PPD-quinone. The MRM transition highlighted in bold was used as quantifier.

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

■ Results and Discussion

The MRM optimization was performed on neat 6-PPD-quinone standard. The product ions during the automatic MRM optimization process matched those published by *Tian et. al*[1]. Three MRM transitions were selected for chromatographic method development (Table 1c). Figure 1b shows an example MRM chromatogram for the quantifier ion *m/z* 299.20>241.15 at 1 ng/mL in neat standard.

Figure 1a shows excellent linearity observed for 6-PPD-quinone in the selected 0.01-100 ng/mL range (r^2 >0.999, weighting 1/c). The percent accuracy for the triplicate injections of calibrants ranged from 85.3 to 122.3% (average % accuracy 91.9-110.6) (Table 2).

Water from a local stream and Savage River in Columbia, MD was collected and spiked at 0.1, 1, 10, and 50 ng/mL 6-PPD-quinone. Figure 2a shows the overlay of MRM chromatograms for the local stream and 2b shows the Savage River for a 0.1 ng/mL spike. The spike recovery was 100 and 90% at 0.1 ng/mL for the local stream and river, respectively. The overall spike recovery at all other concentrations ranged from 83-100%, demonstrating excellent sensitivity and low to none matrix-related signal suppression (Table 3).

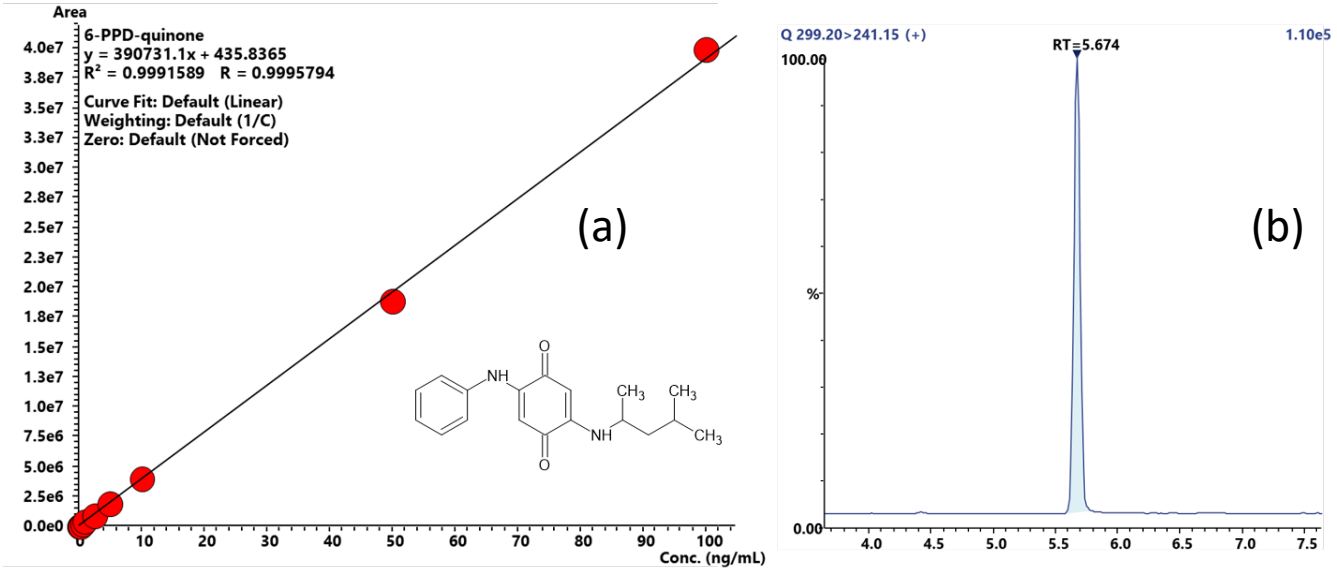

Figure 1: (a) Linear calibration curve for 6-PPD-quinone in the 0.01-100 ng/mL range. Inset: Chemical structure of 6-PPD-quinone. (b) Example MRM chromatogram for 6-PPD-quinone (*m/z* 299.20 > 241.15) at 1 ng/mL neat standard.

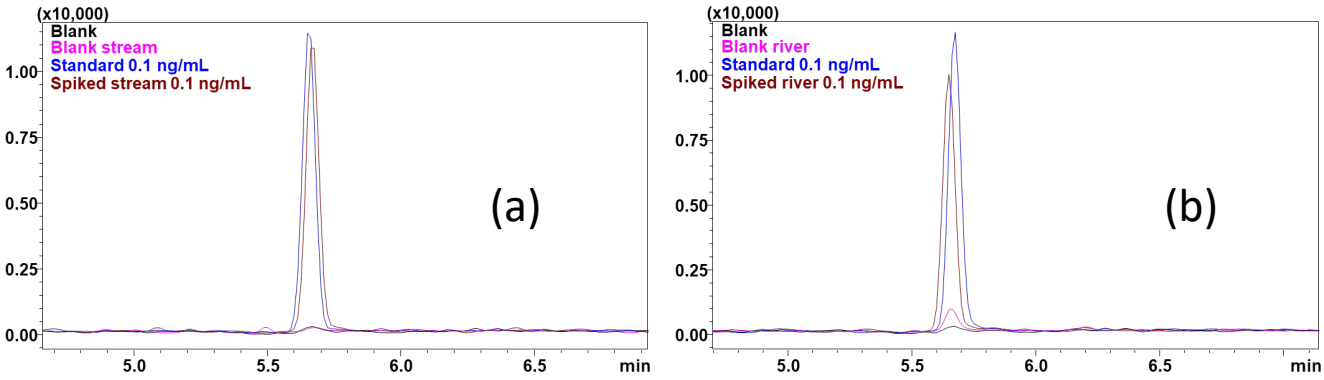

Figure 2: MRM (*m/z* 299.20 > 241.15) overlay of (a) solvent blank, unspiked local stream (blank stream), neat standard at 0.1 ng/mL, and local stream spiked at 0.1 ng/mL (b) solvent blank, river water (blank river), neat standard at 0.1 ng/mL, and river water spiked at 0.1 ng/mL.

Table 2: Area, %accuracy, and %RSD observed for all the different calibration levels.

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

Table 3: Percent recovery of 6-PPD-quinone when spiked into water obtained from a local stream and in Savage river water (Columbia, MD; n=3).

	Local stream	Savage River
Spike level	**% recovery**	**% recovery**
0.1	100.0	90.0
1	95.7	83.2
10	96.7	93.3
50	96.3	98.4

■ Conclusion

A rapid and reliable identification and quantitation method for the analysis of 6-PPD-quinone was developed using the LCMS-8060 instrument.

Excellent sensitivity and linearity (r^2>0.99) were observed in the 0.01-100 ng/mL range. The overall accuracy for neat standards was within 85 to 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. 6-PPD-quinonene was not detected in the stream sample and the concentration was below the calibration range in the river sample.

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.

■ Reference

1. Tian Z, Zhao H, Peter KT, et al. A ubiquitous tire rubber-derived chemical induces acute mortality in coho salmon. *Science*. 2021;371(6525):185-189. doi:10.1126/science.abd6951

ULTRA FAST MASS SPECTROMETRY


LCMS-8040


LCMS-8045


LCMS-8050


LCMS-8060NX


LCMS-2020


Q-TOF LCMS-9030

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