SHIMADZU

Analysis of 6PPD-Q in Reagent and River Water using LCMS-8060RX

WP-269

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1. Overview

A rapid LC-MS/MS method for analysis of 6PPD-Q was developed using the LCMS-8060RX to achieve sufficient sensitivity, accuracy, and reproducibility needed for EPA draft method 1634[1] (EPA 1634) or direct injection methods.

2. Introduction

Contamination of surface water by tire wear particles can dramatically impact ecosystems. Juvenile coho salmon in the US Pacific Northwest were mortally affected from exposure to these tire wear particles from urban stormwater events. Tian et al[2] characterized these contaminants and found that N-(1,3-Dimethylbutyl)-N'- phenyl-phenylenediamine-quinone (6PPD-Q) causes the deleterious effects. N-(1,3-dimethylbutyl)-N′-phenyl-p-phenylenediamine (6PPD) is used ubiquitously as an antioxidant in tire rubber and will oxidize to form 6PPD-Q.

3. Method

6PPD-Q, $^{13}C_6$-6PPD-Q (EIS), and D_5-6PPD-Q (NIS) were obtained from Cambridge Isotope Labs. Standards were prepared in 1:1 H_2O:ACN per EPA 1634[1]. An LCMS-8060RX coupled to a Nexera X3 UHPLC was used for data acquisition. Surface water from an East Coast river and reagent water were analyzed to establish MDLs, precision, and accuracy. Samples were spiked to a 1 ng/mL final concentration of EIS, centrifuged, and a 1-mL aliquot transferred to autosampler vials. NIS was added to a final concentration of 1 ng/mL prior to LCMS analysis.

Table 1. MRM Transitions used for quantitation

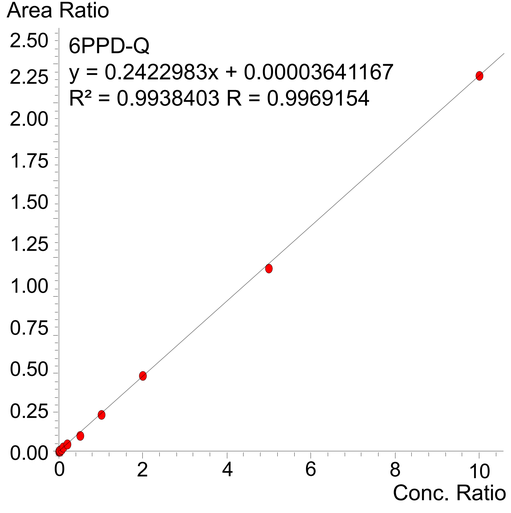
Compound (Polarity)	MRM transition (m/z)	CE	Interface Voltage	Focus Voltage
6PPD-Q (+)	299.18 > 215.05	-19 V	3 kV	3 kV
	299.18 > 241.10	-28 V		
13C6-6PPD-Q (+)	305.20 > 247.10	-31 V	3 kV	0 kV
D5-6PPD-Q (+)	304.21 > 246.1	-28 V	3 kV	1 kV

Table 2. HPLC and MS acquisition parameters

Nexera X3				LCMS-8060RX	
Analytical Column:		Restek Force C18 1.8 µm; 50×2.1 mm		Ionization:	CoreSpray ESI
Mobile Phase	**A:**	0.1% formic acid in water			
	B:	0.1% formic acid in acetonitrile		Interface Temp:	400 °C
Gradient		Time (min)	%B	DL Temp:	200 °C
		0.50	20	Heat Block Temp:	200 °C
		2.00	100	Nebulizing Gas:	7.0 L/min
		3.00	100	Heating Gas:	11.0 L/min
		3.10	20	Drying Gas:	4.0 L/min
		5.00	20	Probe Position:	+1 mm
Oven temp (°C):		40 °C		Interface Voltage:	Varies
Injection volume:		10 µL		Focus Voltage:	Varies

4. Results and Discussion

Figure 1. Calibration curve for 6PPD-Q



The calibration curve of 6PPD-Q displayed excellent linearity from 0.002 to 10 ng/mL (Figure 1). The percent accuracy ranged from 83 to 118% across all calibrants, and the %RSD was <15%. Reagent and river water recoveries ranged from 99-133% and 104-148%, respectively. Unspiked samples did not contain 6PPD-Q at concentrations above the LOQ (Figures 2a and 2b).

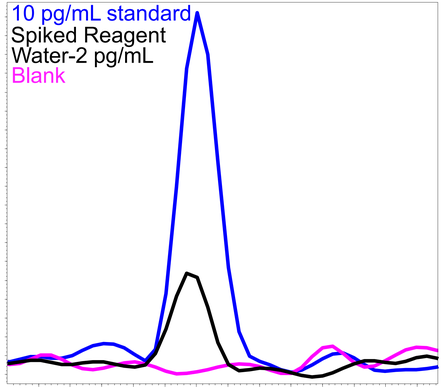


Figure 2a. Reagent water blank, spike, and standard chromatogram

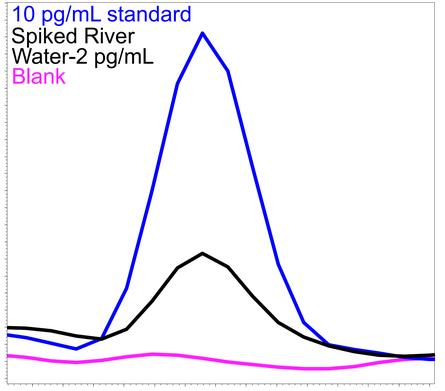


Figure 2b. River water blank, spike, and standard chromatogram.

Table 3. Recovery information for reagent and river water

Nominal Concentration in vial (ng/mL)	Reagent water		River water	
	Average Recovery (%)	Recovery Range (%)	Average Recovery (%)	Recovery Range (%)
0.002	114.1	100.3 - 133.3	135.9	122.4 - 147.8
1	104.1	99.1 - 108.4	107.8	104.3 - 112
10	118.8	115.7 - 122.7	123.1	122.9 - 123.6

5. Conclusion

The system achieved excellent detection performance, including an MDL of 1.1 pg/mL in river water and strong linearity ($R^2 > 0.99$, RSE < 10) across 0.002–10 ng/mL. The method's LOQ was over ten times lower than the requirements in EPA draft method 1634, enabling higher-throughput workflows by reducing the need for extensive sample concentration.

6. Reference

1. U.S. Environmental Protection Agency, Draft Method 1634, Determination of 6PPD-quinone in aqueous matrices using liquid chromatography with tandem mass spectrometry (LC MS/MS), December 2023.
2. 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.