Technical Brief

Quantitation of 6-PPD-Q and Its Metabolites in Fish Using LC-MS/MS

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Tire additive 6-PPD, toxic product 6-PPD-Q, and hydroxylated metabolites were analyzed using this novel method in fish organs.

Introduction

N-(1,3-Dimethylbutyl)-N′-phenyl-*p*-phenylenediamine (6-PPD) is an antioxidant commonly used in rubber tires. Tire abrasion releases it into the environment, where it reacts with ozone to form 6-PPD-quinone (6-PPD-Q). Water runoff carries 6-PPD-Q into streams, where it is highly toxic to aquatic organisms, particularly to certain salmonids, causing widespread mortality and ecological harm.[1] Existing publications report analytical methods for monitoring of 6-PPD and 6-PPD-Q, but not typically the potential metabolites of 6-PPD-Q, because authentic standards have not been available until recently.[2] This study establishes an LC-MS/MS method to measure 6-PPD, 6-PPD-Q, and three hydroxylated metabolites in tissues from freshwater fish in the Great Lakes region.

Methods

Three potential metabolites of 6-PPD-Q were synthesized, purified, and used to evaluate their extraction, chromatography, and mass fragmentation properties to develop a multiple-reaction monitoring (MRM) LC-MS method (**Figure 1**).

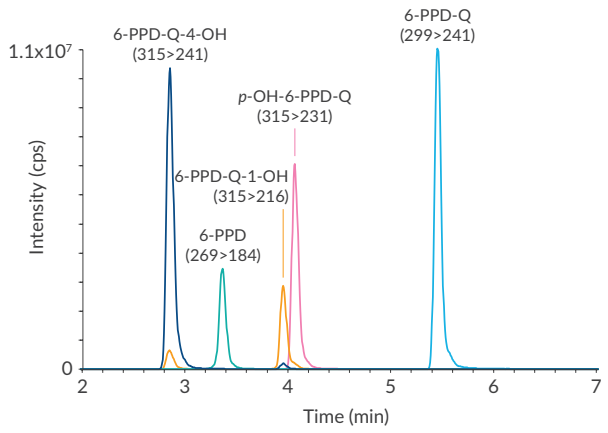

Figure 1 - LC-MS chromatogram of standards showing unique retention times and MS/MS transitions (*m/z*, precursor ion > product ion). Cayman standards used (item numbers in parentheses): 6-PPD-Q-4-OH (42774), 6-PPD (38246), 6-PPD-Q-1-OH (41591), *p*-OH-6-PPD-Q (40605), 6-PPD-Q (38247).

The target analytes were extracted from homogenized fish tissue (100 mg) after protein precipitation in methanol, using internal standard (IS) 6-PPD-Q-d_5 (Item No. 40784). Extracts were injected into an Exion UPLC system coupled to a triple quadrupole 6500+ mass spectrometer (SCIEX). Analytes were separated on a Waters BEH C18 column (50 x 2.1 mm, 1.7 µm, 100 Å) using a gradient of water and methanol, each with 0.1% formic acid and 10 mM ammonium formate. Concentrations were calculated by interpolation of the analyte/IS area ratios into calibration curves prepared with authentic standards.

Results

Of 40 various tissues from 16 fish analyzed, 6-PPD and 6-PPD-Q were detected at low levels in muscle, and at higher levels in other organs (liver, heart, ovaries). Of the three potential metabolites, only *p*-OH-6-PPD-Q was detected, primarily in internal organs of several fish. Representative data for selected analytes and tissues are shown in **Table 1**.

Table 1 - Calculated amounts of analytes in muscle, liver, and heart of a bluegill as representative sample. *NPD* is "no peak detected."

Analyte	LOQ (ng extracted)	Muscle (ng/g)	Liver (ng/g)	Heart (ng/g)
6-PPD-Q	0.001	0.01	0.17	0.35
p-OH-6-PPD-Q	0.0102	*NPD*	2.57	*NPD*
6-PPD-Q-1-OH	0.0914	*NPD*	*NPD*	*NPD*
6-PPD-Q-4-OH	0.0305	*NPD*	*NPD*	*NPD*

Conclusions

This method allows for the simultaneous monitoring of 6-PPD, 6-PPD-Q, and potential metabolites in fish tissue. It can be further extended to include additional PPD compounds and metabolites and could be useful for monitoring water contamination and bioaccumulation.

References

1. Tian, Z., *et al. Science* **371(6525)**, 185-189 (2021).

2. Ankley, P.J., *et al. Environ. Sci. Technol.* **59(28)**, 14214-14225 (2025).