

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

Paul D. Kennedy, Samantha K. Goodwin, Matthew J. Kiewski, Dilrukshika S.W. Palagama, & Miguel A. Gijón

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).

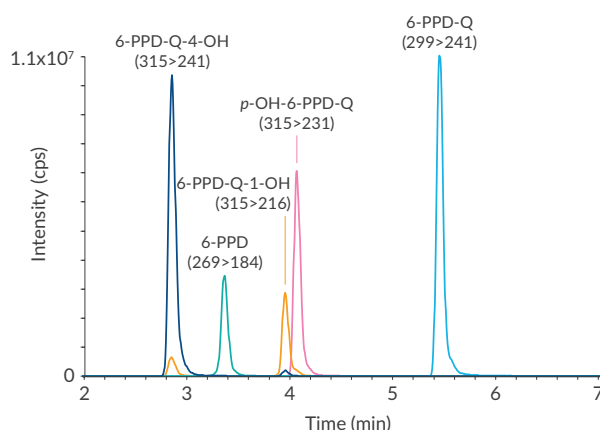


Figure 1 - LC-MS chromatogram of standards showing unique retention times and MS/MS transitions (*m/z*, precursor ion > product ion). Cayman standards used (item numbers in parentheses): 6-PPD-Q-4-OH (42774), 6-PPD (38246), 6-PPD-Q-1-OH (41591), *p*-OH-6-PPD-Q (40605), 6-PPD-Q (38247).

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
<i>p</i> -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

- Tian, Z., et al. *Science* **371**(6525), 185-189 (2021).
- Ankley, P.J., et al. *Environ. Sci. Technol.* **59**(28), 14214-14225 (2025).