



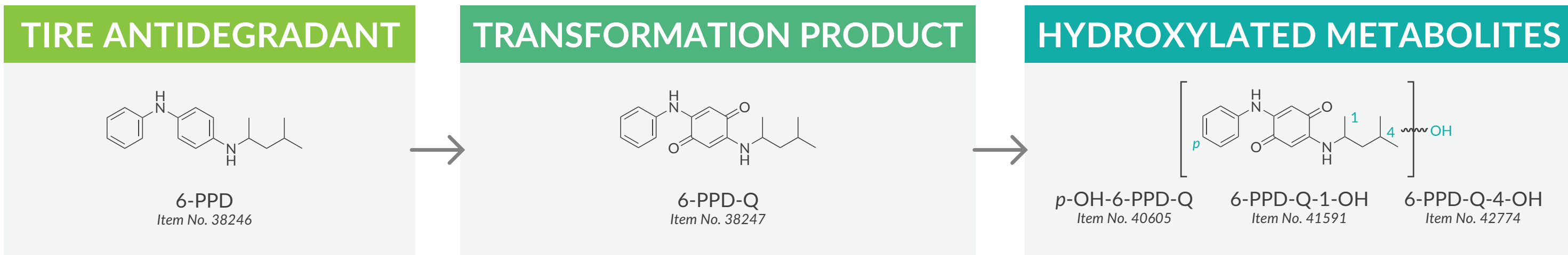
Development of an LC-MS/MS Method for the Quantitation of 6-PPD-Q and Its Metabolites in Fish

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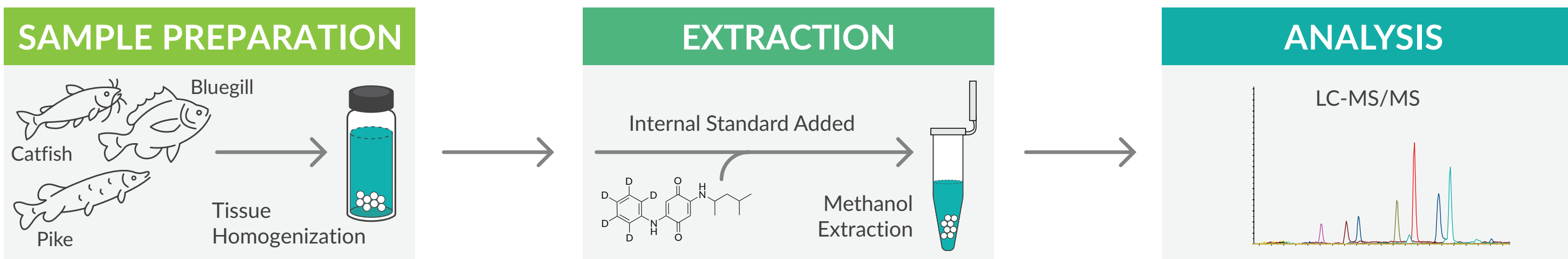
KEY FINDING Tire additive 6-PPD, toxic product 6-PPD-Q, and hydroxylated metabolites, are analyzed using this novel method in fish organs

INTRODUCTION

N-(1,3-Dimethylbutyl)-N'-phenyl-*p*-phenylenediamine (6-PPD) is an antioxidant widely used in rubber tires. Tire abrasion releases 6-PPD which reacts with ozone to form 6-PPD-quinone (6-PPD-Q). *Via* water runoff, 6-PPD-Q reaches streams where it is toxic to aquatic organisms, particularly certain salmonids, contributing to widespread mortality and long-term ecological impacts.¹ Developing robust protocols for quantifying 6-PPD-Q in diverse aquatic organisms as well as in water is essential for environmental monitoring of bioaccumulation and food chain safety. Existing publications report analytical methods for monitoring of 6-PPD and 6-PPD-Q but not typically the potential metabolites of 6-PPD-Q due to the previous lack of availability of authentic standards. This study aims to establish a new method for the extraction and quantitation of 6-PPD, 6-PPD-Q, and three hydroxylated metabolites of 6-PPD-Q in various tissues from freshwater fish in the Great Lakes region.

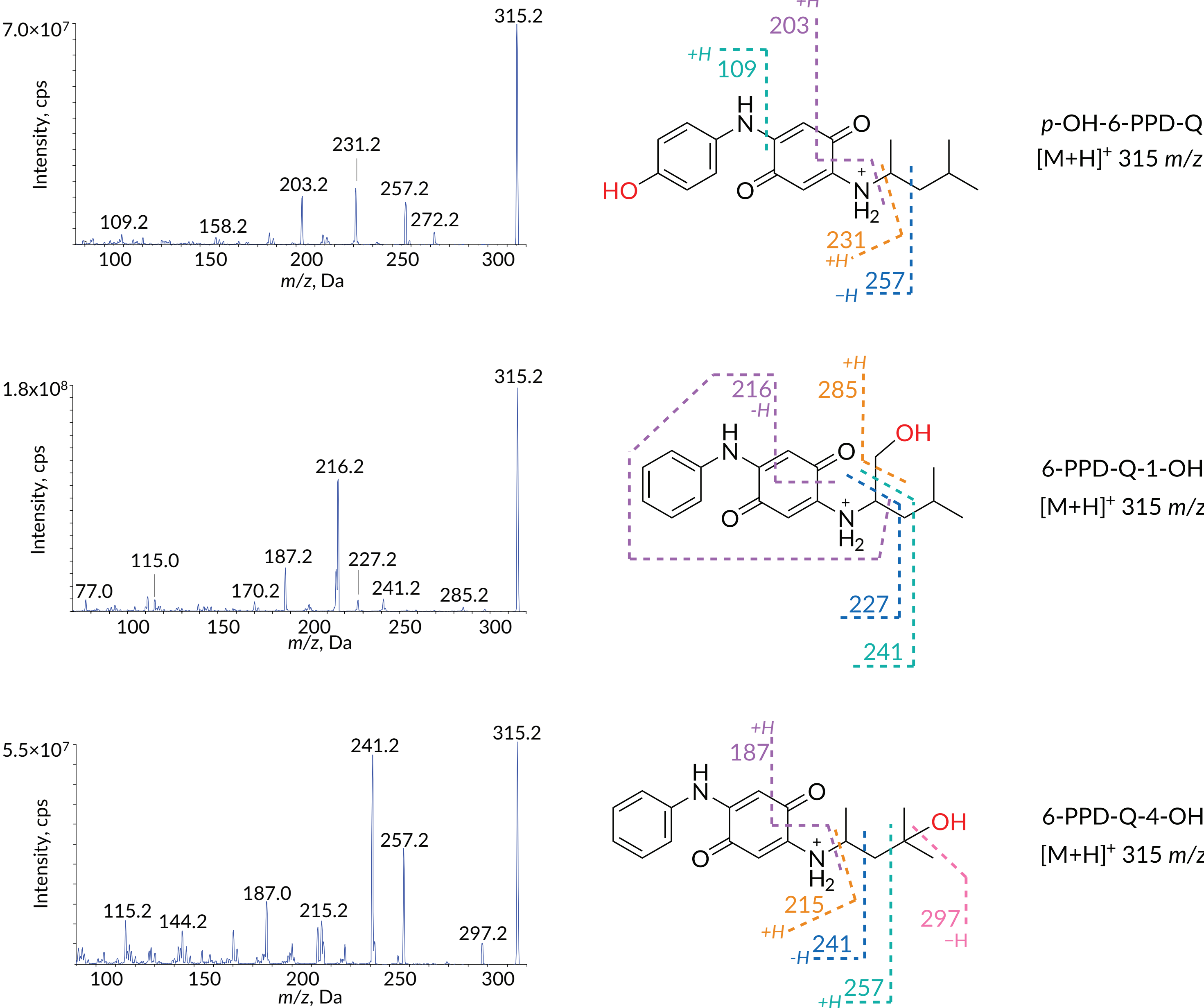


EXPERIMENTAL WORKFLOW



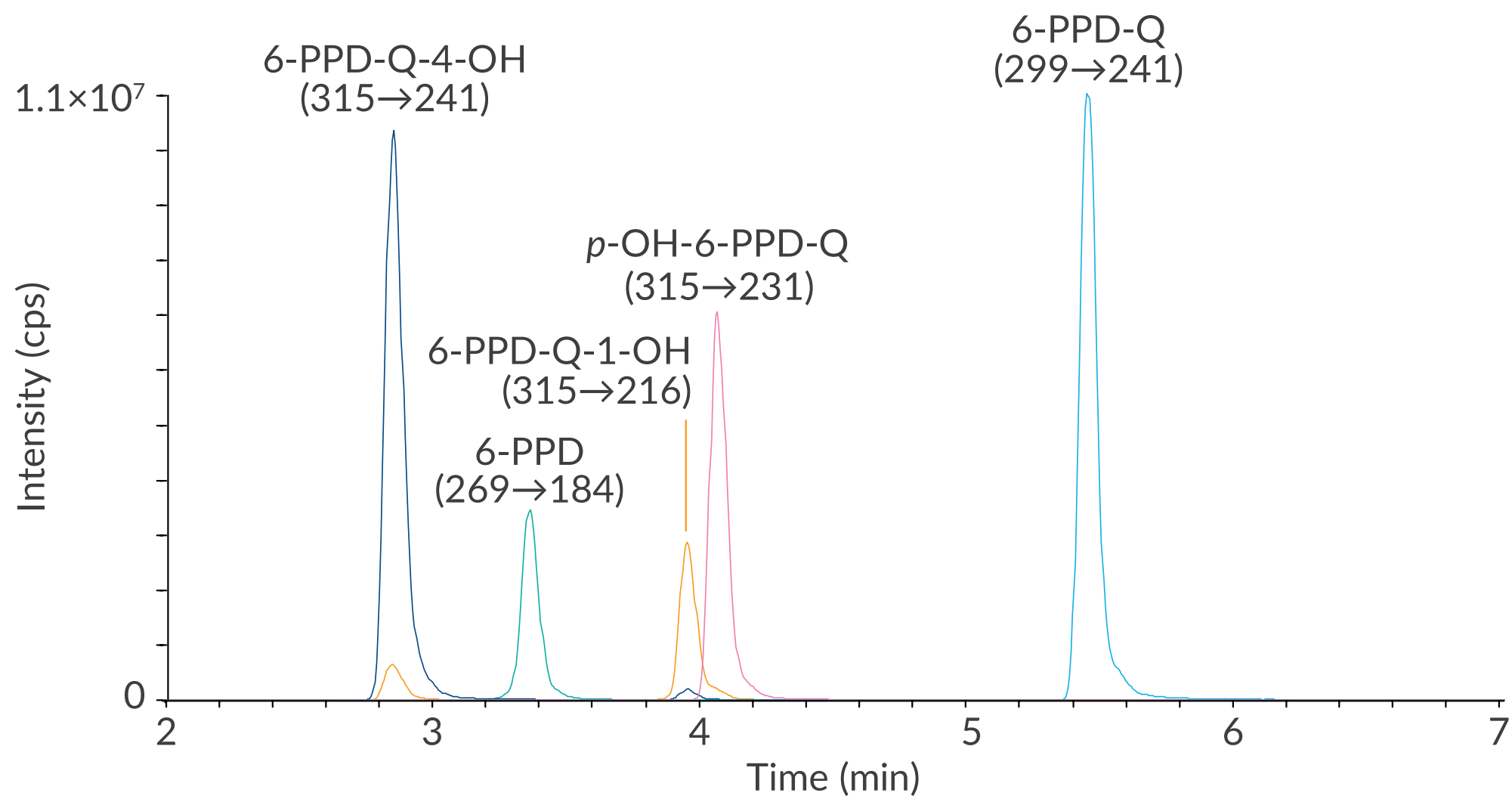
MS/MS

MS/MS spectra of 6-PPD-Q metabolites, and proposed fragmentation²



CHROMATOGRAPHY

Isobaric analytes are resolved by retention time and specific fragments (Q1→Q3)



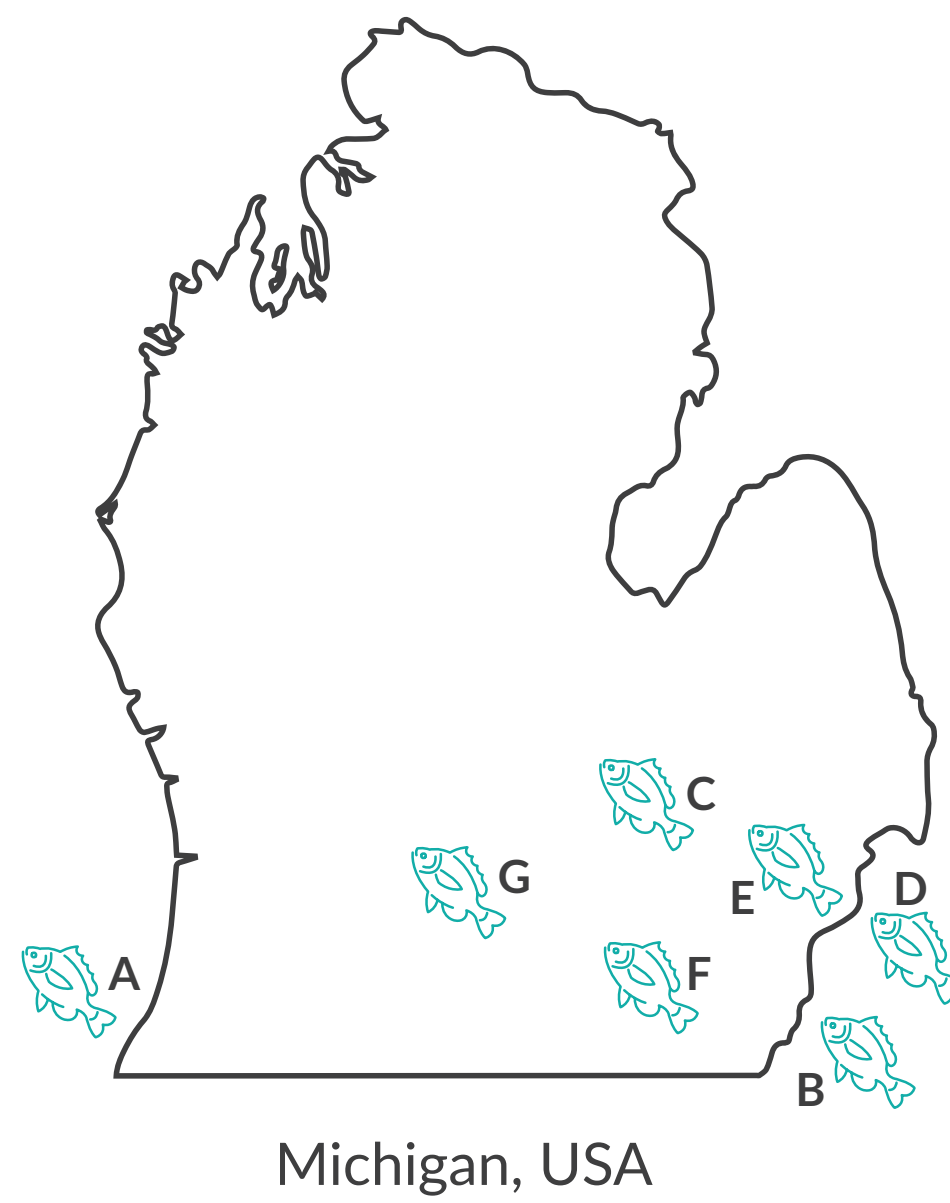
FISH SAMPLE COLLECTION

Fish #	Fish Type	Date Collected	Body of Water	Map ID	Tissue [†]
1	Lake Trout	Summer 2023	Lake Michigan, Benton Harbor, MI	A	M
2	Channel Catfish	Spring 2024	Power Plant, Monroe, MI	B	M, L, O, K
3	Largemouth Bass	Summer 2024	Horseshoe Lake, Whitmore Lake, MI	C	M, T
4	Pike	10/26/25	Detroit River, Detroit, MI	D	M, L, H, O
5	Catfish 1	12/2/25	Argo Dam, Ann Arbor, MI	E	M, L, H, K
6	Shad	12/2/25	Argo Dam, Ann Arbor, MI	E	M, L, K
7	Creek Minnow	12/14/25	Malletts Creek, Ann Arbor, MI	F	W
8	Minnow	12/14/25	Hudson Mills Tributary, Dexter, MI	G	W
9	Minnow	12/14/25	Hudson Mills Tributary, Dexter, MI	G	W
10	Rock Bass 1 & 2 [†]	12/17/25	Argo Dam, Ann Arbor, MI	E	M, L, H
11	Bluegill 1 & 2 [†]	12/17/25	Argo Dam, Ann Arbor, MI	E	M, L, H
12	Catfish 2	2/16/26	Power Plant, Monroe, MI	B	M, L, H, O, K, S

[†] These samples were pools of matched tissues from two fish, due to limited sample collected.

[†] M, muscle; L, liver; H, heart; O, ovary; T, testis; K, kidney; S, swim bladder; W, whole fish.

All fish were collected according to state and local regulations.



EXTRACTION RECOVERY IN MATRIX

Recovery of analytes (20 ng each) spiked into 100 mg muscle from fish #3

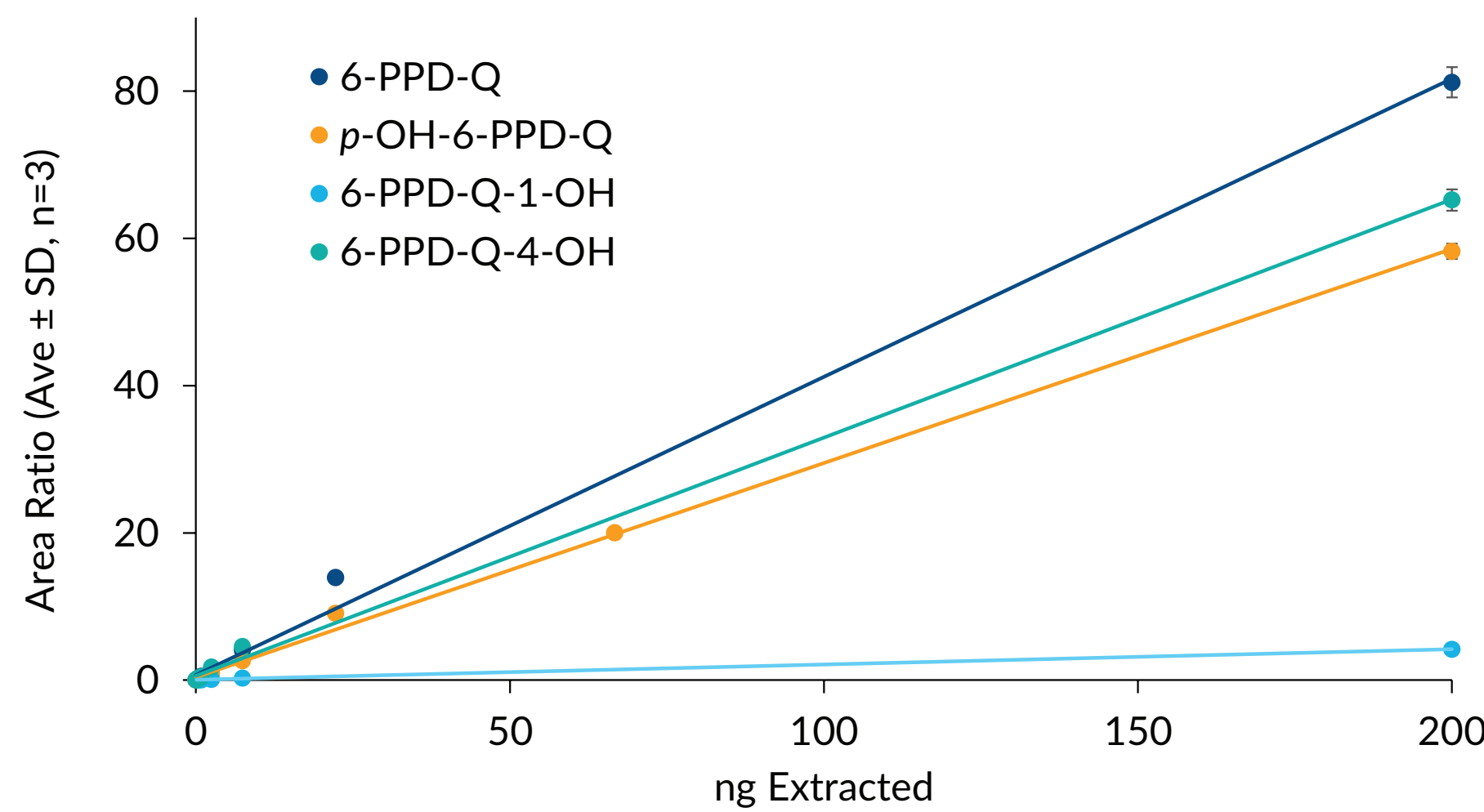
Analyte Name	Extracted in Matrix vs. Extracted in Solvent	
	% Recovery (Ave, n=3)	% RSD (Ave, n=3)
6-PPD*	49.0	17.5
6-PPD-Q	99.2	4.8
p-OH-6-PPD-Q	74.8	16.5
6-PPD-Q-1-OH	101.6	16.4
6-PPD-Q-4-OH	112.6	17.9
Average	97.1	13.9

*The 6-PPD standard was observed to degrade over time in solution.



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CALIBRATION CURVES (EXTRACTED IN SOLVENT)



Analyte Name	Equation	R ²	Linear Range (ng Extracted)
6-PPD-Q	y=0.405x+0.6891	0.9964	0.001–200
p-OH-6-PPD-Q	y=0.2908x+0.4101	0.9981	0.0102–200
6-PPD-Q-1-OH	y=0.0207x+0.0399	0.9992	0.0914–200
6-PPD-Q-4-OH	y=0.3298x+0.556	0.999	0.0305–200

QUANTITATION RESULTS

Fish #	Fish Type	Amount of 6-PPD-Q and (p-OH-6-PPD-Q) in Tissues (ng/g)						
		Muscle	Liver	Heart	Ovary	Testis	Kidney	Whole Fish
1	Lake Trout	BLLOQ (NPD)						
2	Channel Catfish	BLLOQ (NPD)	NPD (NPD)		0.23 (NPD)		0.02 (NPD)	
3	Largemouth Bass	BLLOQ (NPD)				NPD (NPD)		
4	Pike	BLLOQ (NPD)	BLLOQ (NPD)	NPD (NPD)	BLLOQ (0.33)			
5	Catfish 1	BLLOQ (NPD)	NPD (NPD)	NPD (NPD)			NPD (NPD)	
6	Shad	NPD	(3.17) BLLOQ (NPD)				NPD (NPD)	
7	Creek Minnow							0.2 (12.43)
8	Minnow 1							0.09 (NPD)
9	Minnow 2							0.01 (NPD)
10	Rock Bass 1 & 2	0.01 (NPD)	0.27 (2.68)	0.09 (NPD)				
11	Bluegill 1 & 2	0.01 (NPD)	0.17 (2.57)	0.35 (NPD)				
12	Catfish 2	BLLOQ (NPD)	NPD (0.22)	NPD (NPD)	0.01 (NPD)		BLLOQ (NPD)	

	The tissue was not collected.	BLLOQ	A peak was detected, but it was below the lower limit of quantitation.
	A peak detected above the limit of quantitation.	NPD	No peak detected.

CONCLUSIONS

- This work represents preliminary steps to develop and optimize a method for the extraction and LC-MS/MS analysis of 6-PPD-Q and three hydroxylated metabolites.
- This method allows for the quantitation of environmental contaminant 6-PPD-Q alongside some of its metabolites in fish tissues to monitor bioaccumulation. This can be a useful tool in studies of aquatic environments and toxicology in the food chain and in humans.
- 6-PPD-Q was detected at low levels in the muscle tissue of many fish and at higher levels in various organs.
- Of the three potential metabolites, only p-OH-6-PPD-Q was detected, primarily in internal organs of several fish. No 6-PPD-Q-1-OH or 6-PPD-Q-4-OH were detected in any sample.
- Future directions include evaluating in more depth the precision, accuracy, and recovery in different tissues to establish the figures of merit of the method, performing more extensive sampling, and analyzing matched samples of both water and fish tissue samples.

References

- Ankley, P.J., da Silva Junior, F.C., Hunnig, B., et al. The Xenometabolome of Early-life stage salmonids exposed to 6PPD-quinone. *Environ Sci Technol.* 2025. **59**(28):14214-14225. doi:10.1021/acs.est.5c01442
- Tian, Z., Zhao H., Peter, K.T., 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