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Analysis of 6PPD-Quinone in Salmon: A Simplified Sample Prep

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Introduction

A recently identified compound, 6PPD-quinone (6PPD-Q), shown in Figure 1, has been linked to Coho salmon mortality as they return to spawn in near-urban creeks. 6PPD-Q is an ozonation product of 6PPD, a stabilizing additive in rubber tires used to prevent degradation and cracking, and it's a common component in road dust.

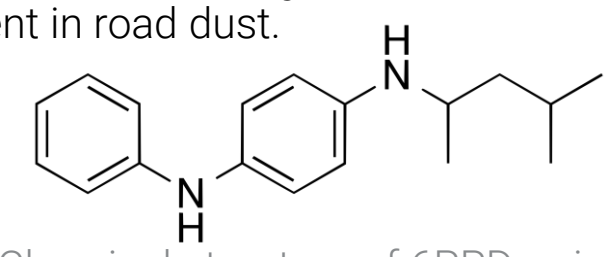

Figure 1. Chemical structure of 6PPD-quinone

Previous experiments have demonstrated acute toxicity at trace levels in roadway runoff contaminating spawning grounds, and salmon mortality rates have been correlated with both road density and traffic intensity. 6PPD also is present in other rubber products such as footwear, synthetic turf infill and playground surfaces so the development of simple workflows to monitor this compound is critical.

Agilent 6475 LC/TQ.

Experimental

Sample Prep

Tissue from store-bought salmon was homogenized and 2 g of material was weighed into a 50 mL tube. Five mL of cold acetonitrile was added to the tube and shaken. Samples were centrifuged for 5 minutes at 5000 rpm, and the supernatant was transferred to a clean tube. The extraction with ACN was repeated, the supernatants combined, and 2.5 mL of water added. The tube was gently inverted to mix. An aliquot of the mixture was then loaded onto the 300 mg/3 mL Captiva EMR-Lipid cartridge and allowed to flow through under gravity. This was followed by a 625 µL rinse of 80:20 ACN:water, which was also collected. The combined eluate was dried down and reconstituted in 1 mL of mobile phase starting conditions for injection onto the LC/TQ (Figure 2).

Experimental

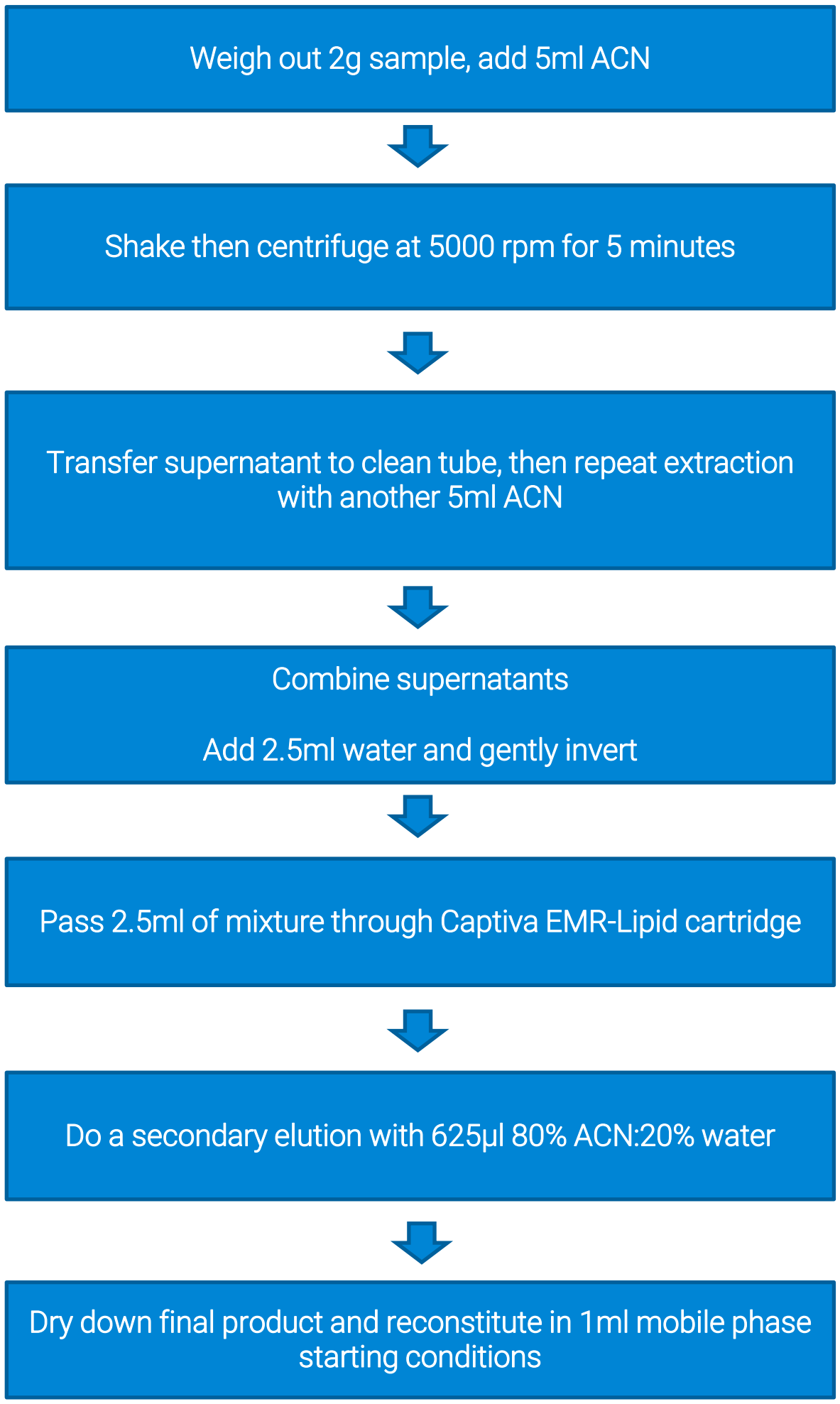

Figure 2. Process schematic for salmon extraction and Captiva EMR-Lipid filtration protocol.

LC-MS/MS Analytical Method

The LC-MS/MS system consisted of a 1290 Infinity II binary pump, a thermostatted multisampler, a temperature-controlled column compartment, and a 6475 triple quadrupole mass spectrometer. Gradient conditions are given in Table 1, with a total injection cycle time of approximately 8 minutes.

Table 1. LC parameters.

Analytical Column	Agilent Poroshell 120 EC-C18, 2.1x50mm, 1.9 µm	
Injection Volume	5 µL	
Mobile Phase A	1 mM ammonium fluoride in water	
Mobile Phase B	Acetonitrile	
Needle Wash	50:20:20:10 Isopropanol:Methanol:Acetonitrile :Water	
Autosampler Temp	4 °C	
Column Temp	40 °C	
Flow Rate	0.3 mL/min	
Gradient	Time (min) 0.00 0.50 4.70 4.80 5.70 5.71	%B 30 30 70 98 98 30
Stop Time	7.50 min	
Post Time	Off	

Table 2. Agilent JetStream ESI source parameters.

Gas Temp	300 °C
Gas Flow	10 L/min
Nebulizer Pressure	40 psi
Sheath Gas Temp	375 °C
Sheath Gas Flow	11 L/min
Capillary Voltage	2500 V
Nozzle Voltage	0 V

Detection of the analytes was undertaken in multiple reaction monitoring (MRM) mode, and the phospholipids were monitored for background levels. Source conditions for the mass spectrometer are shown in Table 2, while MRM conditions are in Table 3. Data was acquired and analyzed using MassHunter software version 12.1.

Experimental

Table 3. MRM transitions monitored for analyte detection as well as phospholipid background.

Compound	Precursor	Product	Frag	CE
6PPD-Q	299.2	241.1	120	33
	299.2	215	120	15
	299.2	187.1	120	29
6PPD-Q-D5	304.2	246.1	130	33
	304.2	220.1	130	15
	304.2	192.1	130	33
PL	184	184	120	0

Results and Discussion

Method Development

Development work demonstrated that the Captiva EMR-Lipid filtration system for sample preparation significantly simplified the workflow while still allowing for excellent detection and recovery of 6PPD-Q. This is due to the unique selectivity of the Captiva EMR-Lipid device for unbranched alkane chains, which differentiates between phospholipids and 6PPD-Q, capturing the former while allowing the latter to pass through the sorbent bed unretained. The phospholipid background was significantly reduced when implementing the EMR filtration prep, compared to just an organic extraction, as shown in Figure 3.

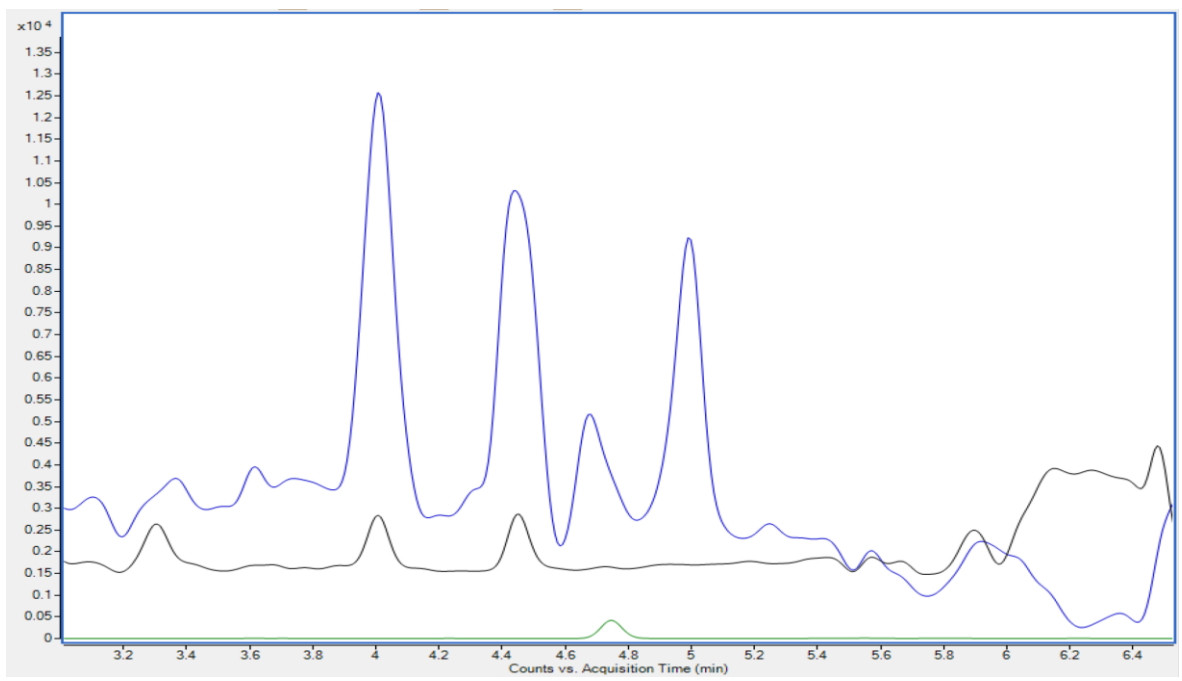

Figure 3. Overlaid chromatograms showing phospholipid background of the Captiva EMR-Lipid prep (black) compared to the ACN extraction with no EMR prep (blue). Green peak shows where 6PPD-Q elutes in the gradient to give a point of reference for background reduction.

Results and Discussion

Previous salmon experiments had suggested a workflow that included ethyl acetate (EtAC) as a part of the extraction solvent system; however, preliminary data showed that there was significant background that resulted from the addition of this solvent that offset any increase in recovery, as shown in Figure 4.

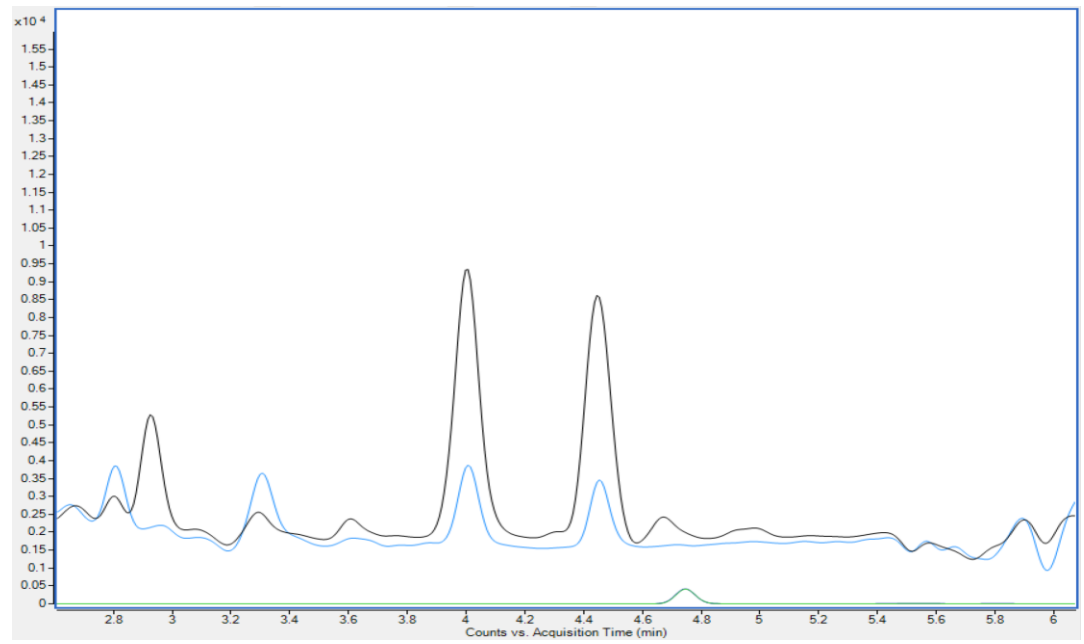

Figure 4. Overlaid chromatograms showing phospholipid background of the Captiva EMR-Lipid prep with an EtAC extraction (black) compared to the ACN extraction (blue). Green peak shows where 6PPD-Q elutes in the gradient to give a point of reference for background reduction.

Chromatography

Chromatography was developed to separate out the target analyte from the phospholipid background. Due to its hydrophobicity, 6PPD-Q elutes in a region with a high phospholipid load (Figure 3 blue trace), and while the Captiva EMR-Lipid protocol helped mitigate that issue, the gradient was designed to ensure that there would be little to no suppression of the trace-level compound from the remaining background (Figure 4).

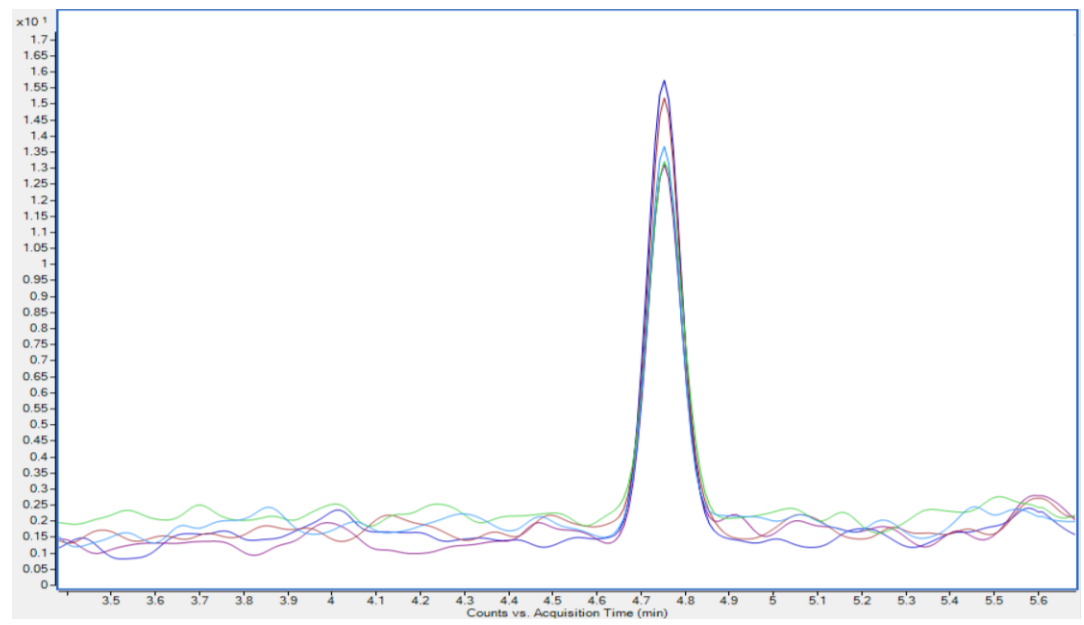

Figure 5. Overlaid chromatograms showing 6PPD-Q reproducibility at LOQ concentration of 3.1 pg/g.

Results and Discussion

The target concentration range for 6PPD-Q was based on previous research published by Tian *et al*., and it was determined that the LOD was 3.1 pg/g, with an RSD of 11.4% over 5 replicate injections (Figure 5). The calibration range in salmon was 10 pg/g-1000 pg/g (Figure 6), with an R^2 of 0.9987, 1/x weighting, and a linear curve fit.

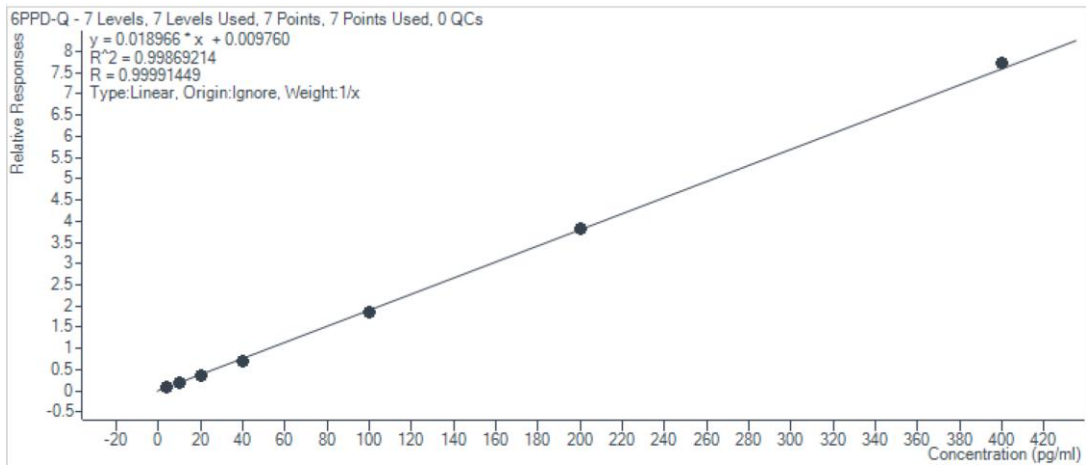

Figure 6. Calibration curve for 6PPD-Q.

Conclusions

- The Captiva EMR-Lipid workflow significantly reduces phospholipid background, allowing for trace-level detection of 6PPD-Q in salmon
- An extraction protocol utilizing ACN instead of EtAC demonstrated similar recoveries while further reducing the phospholipid contribution
- The calibration range was 10-1000 pg/g in salmon, with an LOD of 3.1 pg/g
- Future work includes testing the possibility of increasing throughput via multiplexing on the StreamSelect, given there's only one analyte and one ISTD with a lot of extra idle time
- Future work will also explore automation potential of the workflow

References

- Acute Toxicity of the Tire Rubber-Derived Chemical 6PPD-quinone to Four Fishes of Commercial, Cultural, and Ecological Importance; Brinkmann, M. et al; *Environmental Science & Technology Letters* 2022, *9* (4), 333-338.
- 6PPD-Quinone: Revised Toxicity Assessment and Quantification with a Commercial Standard; Tian, Z. et al; *Environmental Science & Technology Letters* 2022, *9* (2), 140-146.

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