# Nontarget and suspect screening analysis of samples containing compounds derived from tire rubber

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# Why 6PPD-quinone?





### 6PPD ≠ 6PPD-Q

 PPD compounds added to tires to achieve safety standards

 6PPD and other PPDs are added to tire components during manufacturing up to percent level composition and can vary by tire component

 6PPD-quinone is <u>not</u> added to tires and only forms in the process of protecting the tire from oxygen





6PPD

6PPD-quinone

<sup>-</sup>University of Washington; Z. Tian et al.

# LC-HRMS workflows





### SCIEX The Power of Precision

### Quantification method highlights

- LLOQ of 10 ppt detection of 6PPD-quinone and 500 ppt of HMMM
- Samples were passed through a 0.2  $\mu m$  PTFE before being directly injected
- 6 min injection-to-injection time





# 6PPD-Q and HMMM analysis in runoff impacted water



Matrix effects

• 6PPD-Q was spiked in triplicate samples at 50 ppt and 100 ppt



$$SSE (\%) = \frac{Area(spiked extract)}{Area(standard)} \times 100$$

SSE mean values ranged from 93% to 113% for all matrices and spiked concentrations



Spiked concentration



### HMMM was found at **1.6 ± 0.2 ppb** in the parking lot runoff samples



# LC-HRMS workflows





# Typical nontarget workflow





# Communicating confidence





# Communicating confidence





# Nontarget project objectives















Tian, Zhenyu, et al. "A ubiquitous tire rubber-derived chemical induces acute mortality in coho salmon." Science 371.6525 (2021): 185-189.

SCIEX OS software Workflow

# Create a processing method for each fragment within each SWATH acquisition window

Name	Chemical Formula	Precursor (Q1) Mass (Da)	Fragment (Q3) Mass (Da)	XIC Width (Da)	Retention Time Mode	Retention Time (min)	IS Name	Experiment Index
29 (49.436)		110	77.0384	0.02	Find 5 peaks			3 +TOF MSMS (50 - 5000)
29 (30.049)		110	77.0384	0.02	Find 5 peaks			3 +TOF MSMS (50 - 5000)
29 (35.995)		110	77.0384	0.02	Find 5 peaks			3 +TOF MSMS (50 - 5000)
39 (49.392)		175	94.0649	0.02	Find 5 peaks			4 +TOF MSMS (50 - 5000)
39 (45.175)		175	94.0649	0.02	Find 5 peaks			4 +TOF MSMS (50 - 5000)
43 (48.032)		175	159.0916	0.02	Find 5 peaks			4 +TOF MSMS (50 - 5000)





### • 1 unique feature was Identified



- 0.9 ppm error on the fragment





But where are the 215 and 187 fragments?

# Homologous series prioritization of compounds



# Nontarget screening







# Nontarget screening - formula matching



### Next step: SIRIUS and CSI:FingerID



Kai Dührkop, Markus Fleischauer, Marcus Ludwig, Alexander A. Aksenov, Alexey V. Melnik, Marvin Meusel, Pieter C. Dorrestein, Juho Rousu and Sebastian Böcker SIRIUS4: a rapid tool for turning tandem mass spectra into metabolite structure information *Nat Methods*, 16, 2019

Kai Dührkop, Huibin Shen, Marvin Meusel, Juho Rousu and Sebastian Böcker Searching molecular structure databases with tandem mass spectra using CSI:FingerID Proc Natl Acad Sci U S A, 112, 2015.



- Fragmentation pattern analysis:
- Step 1: Input MS1 and MS/MS spectra
- Step 2: Compute all formulas for fragments with masses close to peaks in MS/MS spectrum
- Step 3: Generate a fragmentation tree that best fits the data
- Step 4: Score and rank formulas

# Nontarget screening – formula matching





Processing additives for natural and synthetic rubber in manufacturing tires and other molded and extruded products

Observed mass (Da)	Formula	PPM error	SIRIUS tree intensity explained	Compound name	Level		
280.2635	C <sub>18</sub> H <sub>33</sub> NO	-1.7	84%	Linoleamide	2b		
282.2792	$C_{18}H_{35}NO$	0.20	85%	Oleamide	2a		
310.3024	$C_{20}H_{39}NO$	2.5	91%	Gondamide	2b		
338.3417	$C_{22}H_{43}NO$	0.1	100%	Erucamide	2a		

Darent compounds

### Most intense common fragments



Mass (Da)	Formula	Avg. PPM error
69.07	C5H8	1.3
81.07	C6H8	-1.8
95.09	C7H10	-0.2
149.13	C11H16	2.6

# Nontarget screening – spectral matching





### Library spectra from m/zCloud

### Library spectra from the NIST Tandem (MS/MS) Library from SCIEX



# Nontarget screening – spectral matching



Level 2a

### Stearamide -C18H37NO



**Acquired data** 

### Library spectra from m/zCloud

### Library spectra from the NIST Tandem (MS/MS) Library from SCIEX





The Power of Precision

# Summary





# Summary





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- tire rubber
- Next steps are to purchase standards and comb the sample using a diagnostic ion screening approach for commonly observed fragments of the detected amides

compounds were found in the sample derived from

In addition to 6PPD-quinone, 17 amide

 SCIEX OS software gives the user the flexibility needed to efficiently process nontarget data from complex samples







The Power of Precision

# Thank You



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