Nontarget and Suspect Screening Analysis of Samples Containing 6PPD-quinone and Other Tire-derived Compounds

Karl Oetjen, PhD[1] and Bryan Tackett, PhD[2]
[1]AB Sciex LLC, 500 Old Connecticut Path, Framingham, MA 01701, USA
[2]Phenomenex Inc., 411 Madrid Ave., Torrance, CA 90501, USA

Introduction

Recycled tire products pose risk to both the environment and human health.[1] A recent study identified the presence of a quinone transformation product of N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD), as a potential source of acute mortality in U.S. Pacific Northwest Coho salmon (Oncorhynchus kisutch). 6PPD is used as a tire rubber antioxidant and it, along with its transformation product 6PPD-quinone, were found in roadway runoff and stormwater samples at toxic concentrations.

While roadway runoff is one pathway for tire-related compounds to enter the environment, there are many other ways these compounds can be introduced. Recycled tire products have been used for a variety of purposes including landscaping, rubberized asphalt, and as a synthetic alternative for turf fields. These synthetic turf fields have been installed in the United States since the 1960s and currently, there are 12,000-13,000 synthetic turf fields nationally, with 1,200 – 1,500 new fields being installed each year. According to the United States Environmental Protection Agency (US EPA), 6PPD was observed in nontargeted analysis of both recycling plant and synthetic turf field samples with relatively high response area counts, along with many other compounds that were tentatively identified or not identified.

Due to the wide variety of compounds present in tire derived samples (**Figure 1**) and the presence of thousands of unknown features, comprehensive screening methods and comprehensive data collection methods detection are required. In this technical note, a Luna Omega Polar C18 column, coupled to the SCIEX X500B QTOF system and a data independent acquisition approach (SWATH acquisition), were used to acquire high-quality, high-resolution MS/MS data to screen for both known-unknowns and perform nontargeted analysis. In the tire-derived sample, 17 amide compounds were identified in addition to 6PPD-quinone. Of these 17, several compounds were not found in mass spectral databases or in the literature, as they are likely impurities created in the tire production process.
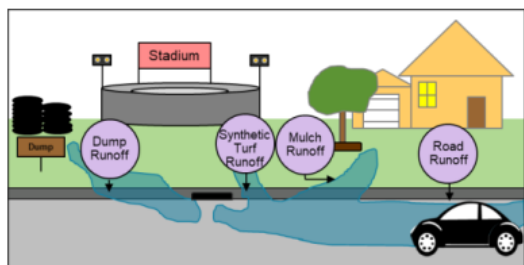
Sample Preparation

Tire derived samples were collected in 10 mL PTFE sampling bottles and passed through a 0.2 µm PTFE syringe filter before analysis.

LC Conditions

Column:	Luna™ Omega 3 µm Polar C18
Dimensions:	100 x 3.0 mm
Part No.:	00D-4760-Y0
Mobile Phase:	A: 0.1 % Formic Acid in Water
	B: 0.1 % Formic Acid in Methanol

Gradient:

Time (min)	%B
0	55
10	55
25	99
34	99
47	99
48	10
56	10

Flow Rate:	0.6 mL/min
Injection Volume:	50 µL
Temperature:	40 °C
LC System:	SCIEX® ExionLC™
Detection:	TOF MS
Detector:	SCIEX X500B QTOF

MRM Conditions

Ion Source:	TurboIonSpray	**TOF Start Mass (Da):**	50
Polarity:	Positive	**TOF Stop Mass (Da):**	5,000
Source Temperature:	500 °C	**Accumulation Time:**	0.05 sec
GS1:	40 psi	**Declustering Potential:**	50 V
GS2:	60 psi	**Collision Energy:**	10 V
CUR:	30 psi		
CAD:	10 psi		
ISV:	3000 V		

Data Processing

Processing of MS data was performed using SCIEX OS software 2.1.6 in which blank filtering, library matching, and formula finding were performed. SIRIUS was also used for database searching. SIRIUS uses isotope pattern analysis and fragmentation trees with structural elucidation to provide a likely parent formulas from large databases like PubChem. This combined approach leads to more accurate predictions and identification rates of more than 70 % on challenging datasets.

Results and Discussion

Blank filtering is a crucial step in nontarget analysis to ensure time is not spent on features present in instrument and extraction blanks. In SCIEX® OS software 2.1.6, this can be done using calculated columns and custom filtering (**Figure 2**). During this same step, gap filling can be applied to ensure errors that would occur due to attempting to divide by zero can be eliminated by adding a small area (0.0001) to each feature. In this study, a blank area threshold of 10 was used to determine which features were present only in the tire derived samples. Using this approach, the number of found features decreased from 174 to 34, thus providing 34 features unique to the tire derived samples to be identified.

A suspect list was compiled from the EPA 2019 tire crumb rubber characterization report, and the newly identified 6PPD-quinone was also added.[1] This list contained 111 positively ionized compounds and from this list, two compounds were identified and later confirmed with MS/MS library spectra: 6PPD-quinone (**Figure 3**) and Oleamide. Oleamide is a common processing additive used in manufacturing tires and other molded and extruded products.

The remaining 32 features were processed using a nontarget workflow. The feature list was first sorted by the ratio of the compound in the blank and the area in the samples. This approach was taken to prioritize the features most unique to the samples. From this, formula finding, adduct identification, and NIST '17 MS/MS Library searching were performed using Analytics in SCIEX OS software. Two additional compounds were identified from the NIST '17 MS/MS library search: Stearamide ($C_{18}H_{37}NO$)

and Palmitamide ($C_{16}H_{34}NO$), both common additives in tire production. The results from the formula finding in SCIEX OS software were compared to the formula results from SIRIUS. Matching top formulas were then prioritized and 2 features with a similar formula to Oleamide, differencing by only the number of -CH2- units, were identified, thereby leading to the discovery of Erucamide ($C_{22}H_{43}NO$), another additive used in tires and molded rubber products, and Gondamide ($C_{20}H_{39}NO$) (**Figure 5**).

To further screen the unknown features, a suspect list was built that contained all the theoretically possible homologues of Gondamide and Stearamide differing only by -CH2- units. An example of this approach is shown in **Figure 4**. Here, 7 features are shown to differ only by the number of -CH2- units and have the same retention time shift between homologs. Using this approach, 11 addition features were identified and were confirmed using diagnostic ions, retention time shift, and mass accuracy. Of these 11, several had the same accurate mass and fragmentation, but existed at different retention times due to the existence of cis and trans isomers (**Figure 6**).

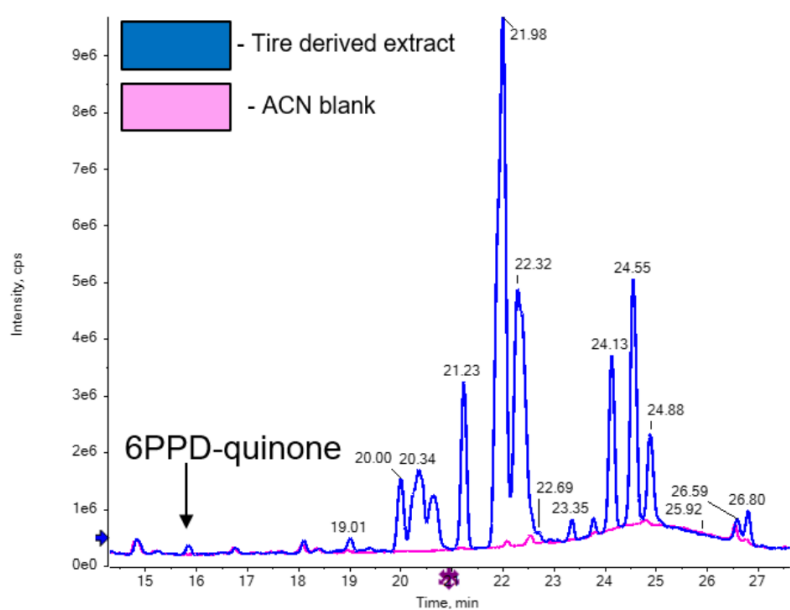**Figure 1.** Total Ion Chromatogram (TIC) of Tested Samples.

Figure 2. Blank Subtraction Workflow in SCIEX® OS Software.

Step 1: Identify the area features present in blanks

Step 2: Calculate the ratio of area found in blank to the area in the sample

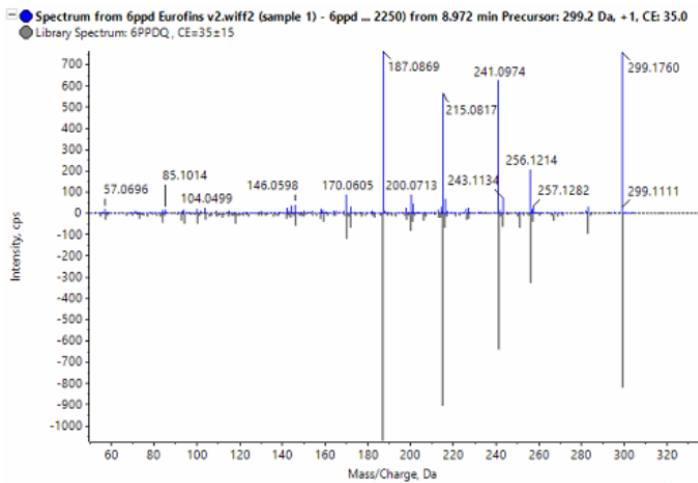**Figure 3.** 6PPD-quinone Sample Versus Library Spectra.

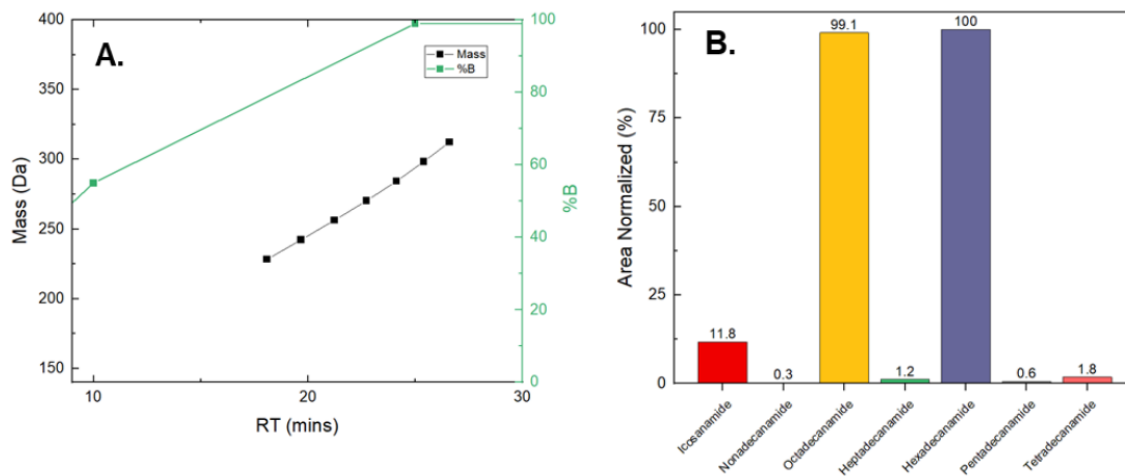**Figure 4.** Homologous Series in Tire-derived Sample.

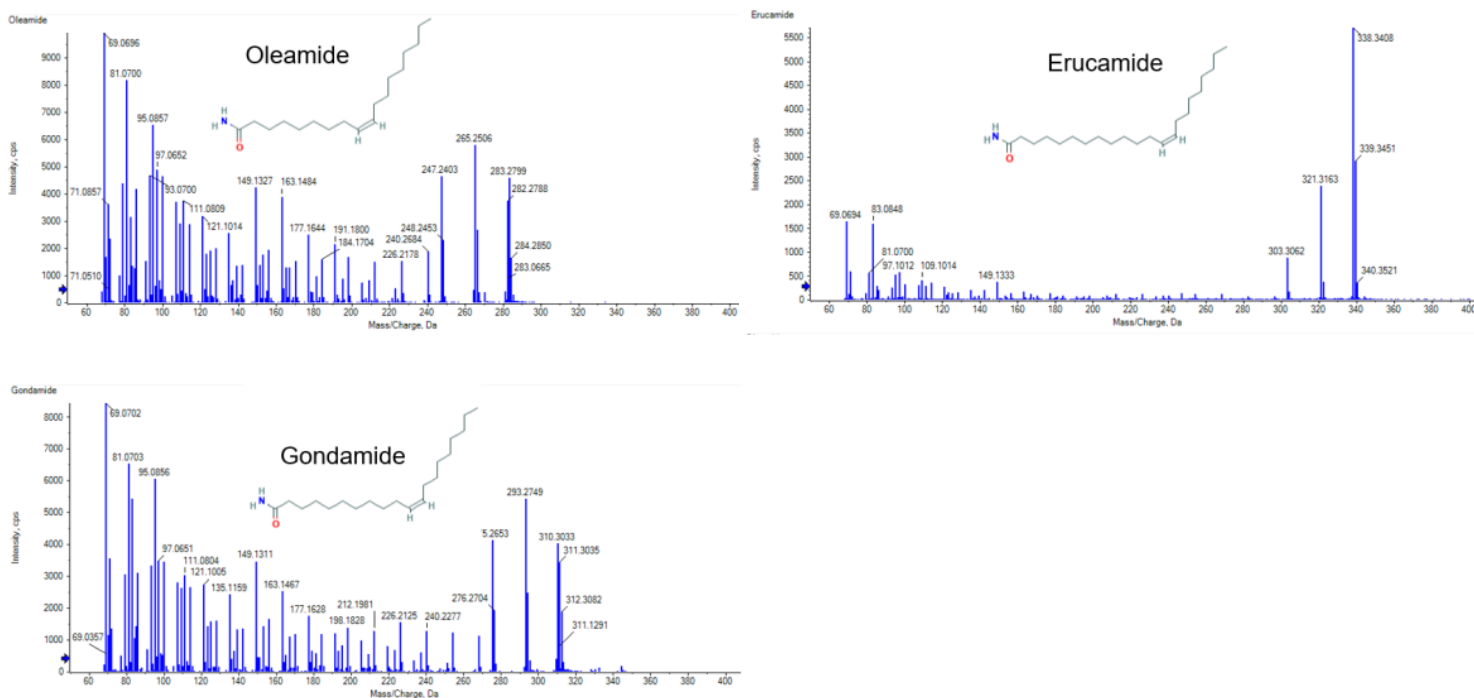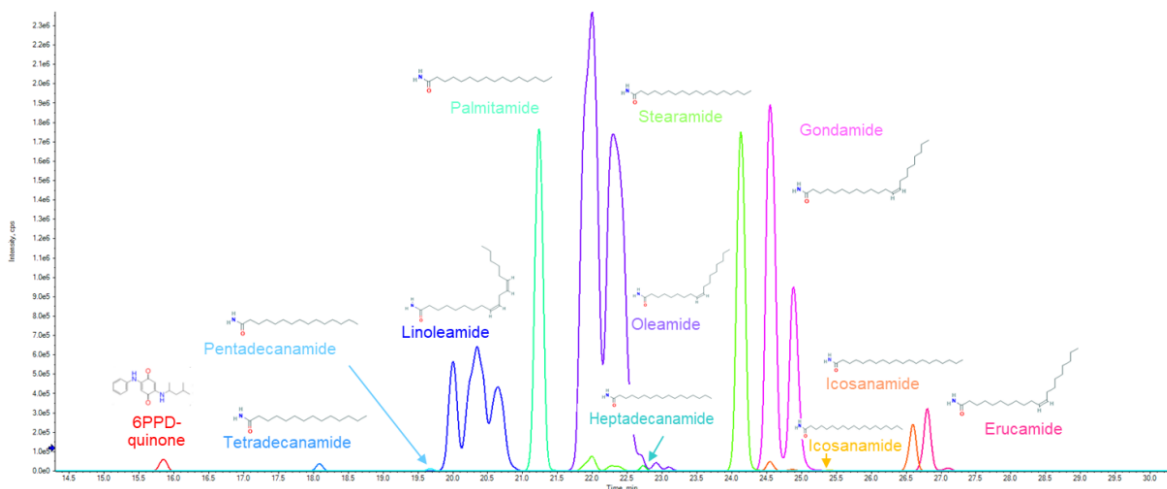**Figure 5.** MS/MS Data for Oleamide, Erucamide, and Gondamide.

Figure 6. Extracted Ion Chromatogram of Identified Compounds in Tire-derived Samples from the Suspect Screening Approach.

Conclusion

As human populations continue to grow and continue to encroach upon aquatic environments, the impact of chemicals derived from tires will continue to increase. These complex mixtures require high resolution LC-MS/MS workflows and advanced software tools for characterization. Using a Luna™ Omega 3 µm Polar C18 LC column along with the SWATH acquisition workflow on the X500B QTOF system and SCIEX® OS software, 18 compounds were discovered in the tire derived samples, including 6PPD-quinone, which has been shown to be extremely toxic to certain salmon species. In addition to 6PPD-quinone, multiple other compounds known to be used during tire production were discovered, while several others are likely impurities present in these known compound mixtures.

Reference

1. United States Department of Environmental Protection. Synthetic Turf Field Tire Crumb Rubber Research Under the Federal Research Action Plan Final Report Part 1 – Tire Crumb Rubber Characterization Appendices; 2019; Vol. 2.

Luna Omega Ordering Information

3 µm MidBore™ Columns (mm)			SecurityGuard™ Cartridges (mm)	
Phases	50 x 3.0	100 x 3.0	150 x 3.0	4 x 2.0*/10pk
Polar C18	00B-4760-Y0	00D-4760-Y0	00F-4760-Y0	AJ0-7600
PS C18	00B-4758-Y0	00D-4758-Y0	00F-4758-Y0	AJ0-7605
C18	00B-4784-Y0	00D-4784-Y0	00F-4784-Y0	AJ0-7611
SUGAR	—	—	00F-4775-Y0	AJ0-4496

for ID: 2.0 – 3.0 mm

*SecurityGuard Analytical Cartridges require holder, Part No.: KJ0-4282

Have questions or want more details on implementing this method? We would love to help!
*Visit **www.phenomenex.com/Chat** to get in touch with one of our Technical Specialists*

Need a different column size or sample preparation format?

No problem! We have a majority of our available dimensions up on www.phenomenex.com, but if you can't find what you need right away, our super helpful Technical Specialists can guide you to the solution via our online chat portal www.phenomenex.com/Chat.

Australia
t: +61 (0)2-9428-6444
auinfo@phenomenex.com

Austria
t: +43 (0)1-319-1301
anfrage@phenomenex.com

Belgium
t: +32 (0)2 503 4015 (French)
t: +32 (0)2 511 8666 (Dutch)
beinfo@phenomenex.com

Canada
t: +1 (800) 543-3681
info@phenomenex.com

China
t: +86 400-606-8099
cninfo@phenomenex.com

Czech Republic
t: +420 272 017 077
cz-info@phenomenex.com

Denmark
t: +45 4824 8048
nordicinfo@phenomenex.com

Finland
t: +358 (0)9 4789 0063
nordicinfo@phenomenex.com

France
t: +33 (0)1 30 09 21 10
franceinfo@phenomenex.com

Germany
t: +49 (0)6021-58830-0
anfrage@phenomenex.com

Hong Kong
t: +852 6012 8162
hkinfo@phenomenex.com

India
t: +91 (0)40-3012 2400
indiainfo@phenomenex.com

Indonesia
t: +62 21 5019 9707
indoinfo@phenomenex.com

Ireland
t: +353 (0)1 247 5405
eireinfo@phenomenex.com

Italy
t: +39 051 6327511
italiainfo@phenomenex.com

Japan
t: +81 (0) 120-149-262
jpinfo@phenomenex.com

Luxembourg
t: +31 (0)30-2418700
nlinfo@phenomenex.com

Mexico
t: 01-800-844-5226
tecnicomx@phenomenex.com

The Netherlands
t: +31 (0)30-2418700
nlinfo@phenomenex.com

New Zealand
t: +64 (0)9-4780951
nzinfo@phenomenex.com

Norway
t: +47 810 02 005
nordicinfo@phenomenex.com

Poland
t: +48 22 104 21 72
pl-info@phenomenex.com

Portugal
t: +351 221 450 488
ptinfo@phenomenex.com

Singapore
t: +65 6559 4364
sginfo@phenomenex.com

Slovakia
t: +420 272 017 077
sk-info@phenomenex.com

Spain
t: +34 91-413-8613
espinfo@phenomenex.com

Sweden
t: +46 (0)8 611 6950
nordicinfo@phenomenex.com

Switzerland
t: +41 (0)61 692 20 20
swissinfo@phenomenex.com

Taiwan
t: +886 (0) 0801-49-1246
twinfo@phenomenex.com

Thailand
t: +66 (0) 2 566 0287
thaiinfo@phenomenex.com

United Kingdom
t: +44 (0)1625-501367
ukinfo@phenomenex.com

USA
t: +1 (310) 212-0555
www.phenomenex.com/chat

⊕ **All other countries/regions
Corporate Office USA**
t: +1 (310) 212-0555
www.phenomenex.com/chat

www.phenomenex.com

Phenomenex products are available worldwide. For the distributor in your country/region, contact Phenomenex USA, International Department at international@phenomenex.com

BE-HAPPY™ GUARANTEE

Your happiness is our mission. Take 45 days to try our products. If you are not happy, we'll make it right.
www.phenomenex.com/behappy

TN55390823_W

*Have questions or want more details on implementing this method? We would love to help!
Visit www.phenomenex.com/Chat to get in touch with one of our Technical Specialists*

phenomenex