Quality Assurance Project Plan

Evaluation of 6PPDQ treatment using soils and sorbent media

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Publication Information

Each study funded by the Washington Department of Ecology ("Ecology") must have an approved Quality Assurance Project Plan (QAPP). This QAPP describes a short-term research project for the Washington Department of Ecology to better understand fate and treatment of 6PPDQ.

This QAPP describes the objectives of the study and the procedures to be followed to achieve those objectives. It is available on request from the authors at University of Washington – Tacoma, Center for Urban Waters (https://www.urbanwaters.org).

Results from this project will be available on request from the authors or Ecology.

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Evaluation of 6PPDQ treatment using soils and sorbent media

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List of Abbreviations

ANOVA	Analysis of Variance
BMP	Best Management Practices
CFS	Cubic Feet per Second
COC	Chain of Custody
CRM	Certified Reference Material
CUW	Center for Urban Waters
DLG	Detection Limit Goals
DQI	Data Quality Indicator
DQA	Data Quality Assessment
DQO	Data Quality Objective
Ecology	Washington State Department of Ecology
EPA	Environmental Protection Agency
HRMS	High Resolution Mass Spectrometry
IQR	Interquartile Range
LC	Liquid Chromatography
LCS	Laboratory Control Sample
LD	Laboratory Duplicate
LIMS	Laboratory Information Management System
MDL	Method Detection Limit
MQO	Measurement Quality Objective
MS	Matrix Spike
MSD	Matrix Spike Duplicate
Permit	NPDES Phase I Municipal Stormwater Permit
QA/QC	Quality Assurance/Quality Control
QAC	Quality Assurance Coordinator
QAPP	Quality Assurance Project Plan
QQQ	Triple Quadrupole Mass Spectrometer
RL	Reporting Limit
RPD	Relative Percent Difference
SOP	Standard Operating Procedure
SR	State Route
USGS	United States Geological Survey
UWT	University of Washington Tacoma
6PPDQ	6PPD-quinone

2.0 Abstract

2-anilo-5-[(4-methylpentan-2-yl)amino]cyclohexa-2,5-diene-1,4-dione is a recently discovered chemical also known as 6PPD-quinone (6PPDQ), that is present in roadway runoff and derived from tire rubber. Recent data indicates that 6PPDQ is primarily responsible for the acute mortality observed in coho salmon in stormwater-impacted watersheds throughout the Puget Sound basin. 6PPDQ is a transformation product of the anti-ozonant N-(1,3-dimethybutyl)-N'-phenyl-p-phenylenediamine (6PPD), which the literature implies is added to all vehicle tire rubbers during manufacturing to prevent rubber cracking and degradation. Notably, 6PPDQ is toxic to juvenile coho salmon at concentrations near ~0.095 µg/L (LC_{50} value from Tian et al. 2022) and is ubiquitous in roadway runoff. Independent government laboratories (USGS Western Fisheries Research Center; John Hansen, personal communication) have reported an LC_{50} value of 85 ng/L for juvenile coho salmon, with the most sensitive individuals in an exposed population perishing at concentrations of 20-30 ng/L 6PPDQ. Given the widespread occurrence of this compound, including at lethal concentrations in roadway runoff and receiving waters during storm events, we need to better understand the ability of existing and commonly available stormwater treatment systems to remove 6PPDQ from roadway runoff and stormwater. Therefore, this research will focus on lab-scale sorbent media-based systems to characterize the partitioning and capacity for 6PPDQ removal under ambient conditions.

The main objectives of this research project are to:

1. Purchase and install an accelerated solvent extraction (ASE) system, then use it to develop an automated method to extract 6PPDQ from soils and treatment media;
2. Evaluate the partitioning (sorption and desorption, kinetics, partitioning coefficients) of 6PPDQ from natural soils representative of urban environments that meet soil suitability criteria or are used for road embankments; and
3. Evaluate the partitioning (sorption and desorption, kinetics, partitioning coefficients) of 6PPDQ from engineered materials and sorbents typically used in stormwater treatment systems.

The first objective will be accomplished by conducting a series of method development experiments, with analysis of 6PPDQ by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS), after acquisition of the ASE system. The latter two objectives will be accomplished by obtaining and/or generating soils and sorbent materials, performing a series of laboratory partitioning and capacity experiments, and then quantifying 6PPDQ by LC-MS/MS.

3.0 Background

3.1 Introduction and problem statement

For over 15 years in the Puget Sound region, researchers have documented urban runoff mortality syndrome (URMS), a phenomenon in which urban stormwater exposure causes widespread acute mortality in 100s-1000s of adult coho salmon (*Oncorhynchus kisutch*) annually (Scholz et al., 2011). Urban stormwater has also been shown to be lethally toxic to juvenile coho salmon (Chow et al., 2019). Coho salmon have tremendous cultural, ecological, and economic value in Washington and they also are a widely accepted sentinel/indicator species for habitat quality in the western US. In addition to substantial value in recreational fisheries, which may exceed commercial fisheries value by over 10-fold, Washington State commercial fisheries averaged $4.3M/year for coho from 2000-2014 ($30.2M/year across all of the US) (NOAA, 2016).

URMS is caused by polluted urban stormwater derived from roadway runoff (Feist et al., 2017; McIntyre et al., 2018), and has more specifically been linked to chemicals derived from tire rubber (McIntyre et al., 2021; Peter et al., 2018). Recently, the primary causal toxicant for URMS was identified as 6PPD-quinone (6PPDQ), a previously unknown ozonation transformation product of the common tire rubber anti-ozonant 6PPD (Tian et al., 2021). Currently, given that 6PPDQ was recently discovered, there exist many data gaps and uncertainties regarding its presence and management in the environment. 6PPDQ is acutely toxic to juvenile coho salmon at concentrations near or below 100 ng/L, with sensitive individuals perishing at concentrations as low as 20 ng/L. Absent current regulations, such concentrations provide quantitative goals for treatment and water quality management efforts. Driven by its widespread use in tire rubbers and ubiquitous presence in roadway runoff (at concentrations up to 100s-1000s ng/L for busy roads), we expect this toxic chemical to occur in all receiving waters downstream of busy roadways (Tian et al., 2021, 2022; Klöckner et al., 2021a), likely at lethal concentrations during some storm events.

Notably, 6PPDQ is a transformation product; it is generated from the reaction of an antioxidant ‘parent’ chemical 6PPD that is added to all vehicle tires. Both 6PPD and 6PPDQ are high risk aquatic toxicants (OSPAR Commission, 2006; Tian et al., 2021). While this new chemical and its suspected provenance have been identified and verified in the scientific literature, considerable research is needed to characterize its environmental transport and fate, its removal in treatment systems and conditions promoting treatment to reduce toxicity that it induces in coho salmon and other species relevant to Washington State waters. This research effort with the University of Washington – Tacoma Center for Urban Waters (i.e. “University”) is expected to improve the basic understanding of the chemical properties and aspects of 6PPDQ formation and treatment with natural soils and engineered sorbents likely present in treatment systems.

Following discovery of the environmental relevance of the tire additive 6PPD and the oxidized byproduct 6PPDQ, Ecology and partners have reviewed and prioritized a list of data gaps. One

research priority is to evaluate and optimize the performance of natural soils and stormwater treatment media with respect to 6PPDQ. Different components of commonly used stormwater treatment medias will be evaluated. Since, 6PPDQ is expected to occur widely wherever tires are used, there will a wide variety of settings where stormwater management may need to occur. Stormwater management is done through the use of best management practices (BMPs) some of which are focused on treatment and use sorbent medias as part of the engineering design. Here, over the coming 6 months, we will embark on simple laboratory scale studies to better understand partitioning and sorption behaviors of 6PPDQ to natural soils and select sorbent media used in stormwater systems.

3.2 Study area and surroundings

The research described here will be conducted in a laboratory setting, but is relevant throughout Washington and across the natural range of coho salmon, which extends from Alaska to the central coast of California. Although they are not as sensitive, 6PPDQ is also lethal to brook trout and rainbow trout, and thus these results also apply to systems where protecting other sensitive salmonids, or ecological health more generally, is of interest. Coho salmon return from the ocean to spawn in freshwater streams in the fall, and the offspring rear in the same streams for up to a year after hatching. Both adults and juveniles are reported to be adversely impacted by 6PPDQ exposure and seem to have similar sensitivities and exposure time scales.

3.2.1 History of study area

Much of the work to understand URMS was done in the Puget Sound area, the second largest estuary in the United States. The streams used by coho salmon throughout the region have been impacted by human development and increasing urbanization, with resulting degradation of both physical and chemical habitat quality. In particular, non-point sources of pollution such as urban runoff are increasingly recognized as a growing threat to water quality (Walsh et al., 2005). Notably, population modeling predicts localized extinction of coho in urbanized watersheds due to the acute toxicity of roadway runoff to coho salmon (Spromberg and Scholz, 2011). Projected increases in human population (Quinn, 2010) are anticipated to increase development, transportation infrastructure, and corresponding contaminant loads in urbanizing watersheds.

3.2.2 Summary of previous studies and existing data

6PPDQ was recently identified as the primary coho salmon toxicant in roadway runoff and urban stormwater (Tian et al., 2021). Since discovery of 6PPDQ, several other researchers have confirmed its environmental ubiquity, with detections in urban surface waters, roadside dust, roadside soils, PM2.5, and human urine (Challis et al., 2021; Huang et al., 2021; Johannessen et al., 2021b, 2021a; Klöckner et al., 2021a, 2021b). Such detections imply relevance across several key environmental compartments beyond roadway runoff and surface water.

To the best of our knowledge, 6PPD, the common anti-ozonant and the parent compound of 6PPDQ, is ubiquitously used in passenger and commercial vehicle tires (0.4-2% by mass) (Babbit, 1978). 6PPD is designed to diffuse through the rubber matrix to the surface, where it preferentially reacts with ozone and other oxidants, forming transformation products such as 6PPDQ (Lattimer et al., 1983; Hu et al., 2022). Industrial literature indicates that 6PPD is prone to form protective surface films during ozonation by oxidative polymerization and/or radical interactions (Huntink, 2003; Lattimer et al., 1983; OSPAR Commission, 2006). These processes may impact availability/release of 6PPD and 6PPDQ from whole tires on vehicles and tire wear particles, with differences across new and used rubbers expected (Wagner et al., 2022). However, although a few studies have examined leaching of 6PPD and other rubber additives (e.g., 1,3-diphenylguanidine) from tire rubbers (Müller et al., 2022; Peter et al., 2020; Seiwert et al., 2020; Unice et al., 2015), there are currently no studies that examine sorption outcomes for these compounds with soils and sorbent treatment media.

3.2.3 Parameters of interest and potential sources

This study will examine the sorption potential of 6PPDQ, a transformation product of the anti-ozonant 6PPD that is used as an anti-ozonant in tire tread and sidewalls, as well as in various other rubber seals and materials (Babbit, 1978; OSPAR Commission, 2006).

In these studies, chemical contaminant analysis will be performed at the UWT CUW laboratory facility utilizing developed liquid chromatography separation coupled to tandem mass spectrometry (LC/MS/MS) detection techniques. The methodology, including stocks and standards preparation, sample, preparation, and analytical approach, is documented in the CUW SOP entitled “Extraction and Analysis of 6PPD-Quinone in Water” (Appendix A).

3.2.4 Regulatory criteria or standards

There are currently no state or federal regulatory standards for 6PPDQ.

4.0 Project Description

The aim of this project is to improve our understanding of the removal of 6PPDQ in typical roadside soils and sorbent media typical of stormwater treatment systems through laboratory experiments and mass spectrometry-based analytical methods. The overall project objective is to ***assess the effects of soils and sorbent composition on the partitioning and transport of 6PPDQ using laboratory scale systems***. This study will utilize targeted liquid chromatography tandem mass spectrometry (LC/MS-MS) for quantitative detection of 6PPDQ in lab samples and systems. To the degree possible, samples and sorbent media will be obtained from regional and state agencies, manufacturers, or commercial suppliers with input from Ecology.

4.1 Project goals

The specific goal of the project is to:

- Assess the partitioning and kinetics of 6PPDQ partitioning to soils and sorbents using laboratory scale systems.

4.2 Project objectives

Specific objectives of the project are to:

1. Purchase and install an accelerated solvent extraction (ASE) system, then use it to develop an automated method to extract 6PPDQ from soils and treatment media;
2. Evaluate the partitioning (sorption and desorption, kinetics, partitioning coefficients) of 6PPDQ from natural soils representative of urban environments that meet soil suitability criteria;
3. Evaluate the partitioning (sorption and desorption, kinetics, partitioning coefficients) of 6PPDQ from engineered materials and sorbents typically used in stormwater treatment systems; and
4. If possible, preliminarily screen sorbent capacity with batch or column studies.

4.3 Information needed and sources

Both new and existing samples and data sets derived from samples that will be used in this study have been or will be collected by our research group according to established SOPs and workflows within CUW and are described in the peer-reviewed literature. These existing studies and samples include studies examining the partitioning of other trace organic contaminants (including structurally similar tire rubber related compounds) to soils (e.g. Tang et al. 2023, Yang et al. 2019) and related leaching studies examining the kinetics and dynamics of 6PPDQ in a solid-water system (e.g. Hu et al., 2022).

4.4 Tasks required

The following tasks will be carried out during this project:

- Purchase and install an accelerated solvent extraction system (e.g., ThermoFisher Extreva system), then use it to develop an automated method to extract 6PPDQ from soils and treatment media;
- Acquire representative soils and sorbent media from local, regional, and state collaborators, interested parties, manufacturers, and commercial suppliers;
- Prepare, standardize, and characterize physical properties (mass, density, surface area, organic carbon content) of soils and sorbent media used in studies;
- Perform a series of laboratory partitioning studies on natural soils and sorbent media following protocols and procedures for batch equilibration methods described in OECD Method 106;
- Apply the CUW LC/MS-MS method to quantify 6PPDQ concentrations in aqueous samples or extracts of soils/sorbents generated during laboratory experiments. Depending on the system, either direct injection or SPE extraction will be used and
- Integrate data and knowledge on the system mass balances, partitioning dynamics, kinetics, and stability of 6PPDQ to inform treatment system selection and potential management practices for 6PPDQ in roadway systems. Data analysis and presentation will follow methods described in Fu et al. 2016, Tang et al., 2023, and Yang et al. 2019.

4.5 Systematic planning process

Systematic planning is reflected in this QAPP, and the associated standard operating procedure included as Appendix A.

5.0 Organization and Schedule

5.1 Key individuals and their responsibilities

Table 1 shows the responsibilities of those who will be involved in this project.

Table 1. Organization of project staff and responsibilities.

Staff	Title	Responsibilities
Morgan Baker Department of Ecology Phone: 360-706-4079	Ecology Project Manager (Client)	Ecology point of contact for contract management. Clarifies scope of the project. Provides review of all deliverables, including the QAPP, budget, and reports.
Edward Kolodziej University of Washington Tacoma, Center for Urban Waters Phone: 253-692-5659	Project Manager / Principal Investigator	Clarifies scope of the project. Project management. Prepares QAPP and budget. Assists with reporting and data interpretation.
Katherine Peter University of Washington Tacoma, Center for Urban Waters Phone: 253-254-7030 x8007	Research Scientist	Writes the QAPP. Reviews QA/QC. Assists with reporting.
Ximin Hu University of Washington Tacoma, Center for Urban Waters Phone: 443-214-9707	Graduate Student	Conducts laboratory studies; analyzes samples, performs data analysis and draft reporting.
Dave Wark University of Washington Tacoma, Center for Urban Waters	Research Scientist	Leads ASE acquisition and associated method development
Brandi Lubliner WA Department of Ecology Phone: 360-407-7140	Quality Assurance Coordinator and Project Technical Reviewer	Reviews and approves the draft QAPP and the final QAPP.

QAPP: Quality Assurance Project Plan

5.2 Special training and certifications

Project staff have a combined >30 years experience analyzing environmental samples by mass spectrometry for organic contaminants. This expertise also includes instrument maintenance

and care as outlined in CUW SOPs (see Appendix A). All lab personnel are required to take laboratory training courses as administered by the U. of Washington Environmental Health and Safety (https://www.ehs.washington.edu/system/files/resources/ehslabsafetytrainmatrix.pdf). There are no additional training or certification required for project personnel above and beyond what is required per the project staff job classifications. All project staff are trained to demonstrate competency in sample processing and data analysis program components.

5.3 Organization chart

Not applicable - See Table 1.

5.4 Proposed project schedule

Table 2. Proposed project schedule for completing laboratory work and reports.

Deliverable	Anticipated start date	Anticipated completion date	Lead Staff
Laboratory Experiments and Analyses			
Completion of QAPP	12/15/2022	1/15/2023	Ed Kolodziej
Approval of QAPP	1/15/2023	2/15/2023	Ecology
Purchase and Install ASE system	12/15/2022	3/1/2023	Dave Wark
Evaluate 6PPDQ partitioning dynamics and kinetics from representative soils	2/15/2023	6/1/2023	Ximin Hu
Evaluate 6PPDQ partitioning dynamics and kinetics from representative sorbent media	3/15/2023	6/1/2023	Ximin Hu
Broader Impacts and Communication			
Progress Report	4/1/2023	4/15/2023	All
Data presentation at MuniCon 2023		4/26/2023	All
Summary Final Report	6/1/2023	6/30/2023	All

5.5 Budget and funding

This study has been funded by the Washington Department of Ecology (Ecology) under an Interagency Agreement (C2300072). The actual implementation is carried out by the University of Washington Tacoma. The estimated project budget is included below in Table 3.

Table 3. Project budget and funding

TASKS	TOTAL PROJECT COST
1 – Project Management and QAPP	$21,361
2 - Equipment and Supplies	$165,506
3 - Evaluation of Partitioning to Soils and Media	$88,941
Total	$275,808

6.0 Quality Objectives

6.1 Data quality objectives

The main DQOs for this project are to evaluate the partitioning kinetics and constants for 6PPDQ sorption/desorption to natural soils and engineered media using batch equilibrium study designs. To obtain high quality datasets of chemical contaminants that meet Measurement Quality Objectives (MQOs; described below), study designs will follow protocols specified by OECD Method 106 (Adsorption-Desorption using a Batch Equilibrium method) and samples will be analyzed using existing liquid chromatography-tandem mass spectrometry (LC-MS/MS) methodologies and approaches (see appendix A).

6.2 Measurement quality objectives

The MQOs for the data to be collected describe the performance metrics and criteria for acceptance that provide the basis for evaluating data quality and usability. They indicate the minimum threshold levels for measures of bias, repeatability, precision, accuracy, and sensitivity that must be associated with the data.

The primary types of quality control samples used to evaluate the quality of the LC-MS/MS based laboratory analyses are spikes (e.g., Laboratory Control Samples), replicates, and blanks (e.g., Method Blanks). The level of QA/QC expected to be attained in these studies are those consistent with standard methods (e.g., EPA 1694) for analysis of trace organics in environmental systems. Protocols for data assessment from LC-MS/MS methods used here will reflect internal QA/QC protocols and workflows established by CUW laboratories for quantification of trace organic contaminants (including 6PPDQ) in environmental samples. These protocols and workflows are described in the CUW Laboratory SOP for 6PPDQ extraction and analysis (Appendix A), which describes sample collection, sample processing, sample analysis, and data handling, data analysis, and data management. MQOs are summarized in Table 4.

6.2.1 Targets for precision, bias, and sensitivity

The MQOs for project results, expressed in terms of acceptable precision, bias, and sensitivity, are described in this section.

6.2.1.1 Precision

Precision is a measure of the repeatability of a set of replicated results, and is considered to represent random error in the measurement process. Poor precision is due to difficulties in obtaining samples under identical conditions (e.g., contamination, variability of lab system conditions during the time replicate samples are collected) or poor sensitivity of laboratory procedures and instrument performance.

Three key aspects are considered to evaluate precision of LC-MS/MS data: instrument tuning and maintenance, repeated injections of reference standards, and laboratory sample replicates (Table 6, Table 7).

- Instrument tuning: Instrument tuning procedures are described extensively in the SOP for 6PPDQ extraction and analysis (see Appendix A);
- Repeated injections of reference standards: The Continuing Calibration Verification (CCV) sample (a mid-point calibration standard) is analyzed periodically through the analytical run to evaluate analytical variability, following QA/QC criteria described in the SOP for 6PPDQ extraction and analysis (Appendix A);
- Sample replicates: Replicates are samples taken from, and are representative of, the same sampling event/system and carried through all steps of the sampling and analytical procedures in an identical manner. Because replicates for this study are expected to be generated from use of identical, triplicate experimental systems, lab replicates will represent identical processing, extraction, and analysis of individually replicated laboratory-scale experimental systems. These replicates are used to assess variance of experimental systems, processing, and analysis, and improve data accuracy. As possible (based on sample mass/volume and instrument capability), a minimum of 3 experimental replicates will be collected for each sampling event/experimental system.

6.2.1.2 Bias

Bias is the systematic or persistent distortion of a measurement process which makes the result non-representative (i.e., the measured parameter is different than its true value in a given sample). Potential sources of bias include sampling and analytical procedures that introduce contamination, instability of samples during transportation and storage, interference from other constituents in the sample matrix, inability of the analytical method to measure all forms of the constituent of interest, and faulty calibration of the measurement process. Blanks are prepared coincident with sample collection and/or subjected to the same experimental conditions as the study samples, and may provide an indication of contamination due to glassware cleanliness, solvent purity, exposure to laboratory surroundings during studies and sample processing, and transfer/carryover from equipment and instrumentation. Method blanks will be prepared in the laboratory and processed in the same manner as experimental samples and will provide information on any bias associated with the sample preparation process. Instrument blanks are solvent-only (e.g., methanol) samples that will be injected throughout the analytical run and within analytical batches to check for potential analytical interferences, contamination, carryover, or bias associated with the instrumentation. Errors of bias are minimized through use of standardized procedures by properly trained staff and identification/mitigation of key steps or surroundings with highest potential for sample contamination.

6.2.1.3 Sensitivity

Sensitivity is a measure of the capability of a method to detect a substance and discriminate between measurement responses representing variable levels of interest. Sensitivity is measured through reporting limit performance, and in a regulatory setting, the method detection limit (MDL) and method quantitation limit (MQL) are often used to describe sensitivity and the concentration above which the laboratory can report with documented precision and accuracy. In the case of LC-MS/MS analyses, the sensitivity of the method can be assessed based on the detector resolving power, the results of repeated injections of analytical standards, and by setting standards for minimum response of analytes. Blank samples (e.g., methanol blanks, method blanks) are also evaluated for 6PPDQ detection with respect to the minimum detection limit, following QA/QC criteria described in the SOP for 6PPDQ extraction and analysis (Appendix A).

6.2.2 Targets for comparability, representativeness, and completeness

6.2.2.1 Comparability

There are no EPA or other regulatory methods developed to assess 6PPDQ concentrations in water or from soil matrices. The CUW SOP that will be followed for sampling, analysis, and data reduction to ensure comparability is “Extraction and Analysis of 6PPD-Quinone in Water” (Appendix A).

6.2.2.2 Representativeness

Representativeness of samples describes the degree to which the data accurately reflect the population from which they were taken. Data representativeness will be ensured by using experimental replicates to the extent possible, evaluating multiple extraction conditions for ASE method development, evaluating sorption to multiple different soils and treatment media to assess variability, and the use of standardized sample processing and analytical procedures.

6.2.2.3 Completeness

Completeness is the proportion of samples collected relative to the total number planned to be collected, and depends on both experimental sampling and the completion of analytical work by the laboratory. The goal of the study is to collect samples and analyze data for at least 95% of the planned sampling events.

Table 4. MQOs for 6PPDQ Analysis

QA/QC Sample	MQO
CCV	Calculated accuracy 70-130% throughout analytical batch, % RSD <30%
Methanol Blank	<MDL
Method Blank	<MDL
LCS and LCSD	Recovery 60-130%; <40% RPD

6.3 Acceptance criteria for quality of existing data

Existing data that will be used in this study to guide experimental method design, include datasets from studies examining the partitioning of other trace organic contaminants (including structurally similar tire rubber related compounds) to soils (e.g., Tang et al. 2023, Yang et al. 2019) and related leaching studies examining the kinetics and dynamics of 6PPDQ in a solid-water system (e.g., Hu et al., 2022). Data generated for these studies followed established SOPs and QA/QC procedures at the CUW laboratories, meets the MQOs stated in Table 4 (or will be flagged to indicate any deviation), and have undergone rigorous internal and external review.

7.0 Study Design

Understanding the treatment removal potential of natural soils and sorbent media across variable runoff conditions is a stormwater management need. The target question for focused research investigation is: how well do these soils and treatment medias reduce concentrations of 6PPDQ and associated roadway derived organic contaminants? The University will oversee the project and take responsibility for completing deliverables within the schedule/timeline. This task consists of the following elements:

- Partitioning to Un-amended soils: Design and conduct preliminary investigations to further our understanding of interphase partitioning of 6PPDQ to 2- 3 natural soil types representative of those found in Washington surface environments. The final soil types will be agreed upon by the University and Ecology, with input and samples from regional stakeholders. Studies will consist of sorption and desorption trials under batch conditions where partitioning kinetics and constants are evaluated. If time allows, soil capacities will be additionally evaluated by converting select batch systems to flow-through columns where breakthrough is evaluated. Study outputs are expected to inform management and removal of these pollutants in natural environments and passive treatment systems with substantial natural components. Note that these two soil types are dependent on partnership for permission to collect site soil.
    - The natural soils for the study would be collected from sites that are considered by local or state partners to meet site soil suitability criteria outlined in Ecology's stormwater manuals.
    - Engineered roadway embankment soils or natural soils used for natural dispersion along highways that would meet the Washington State Department of Transportation's Highway Runoff Manual.
- Partitioning to Engineered Treatment Media: Design and conduct preliminary investigations to further our understanding of interphase partitioning of 6PPDQ to 6-9 engineered sorption media types representative of those found in engineered stormwater treatment systems. The final sorbents will be agreed upon by the University and Ecology, with input from regional and state stakeholders. Studies will consist of sorption and desorption trials under batch conditions where partitioning kinetics and timescales are evaluated. If time allows, media capacities will be additionally evaluated by converting select batch systems to flow-through columns where breakthrough is evaluated. Both batch and flow-through outputs are expected to inform management and removal of these pollutants in engineered treatment systems. The following sorbent media are in order of preference for evaluation in this study:
    1. Stormwater compost that meets Ecology's stormwater manual specifications
    2. Granular activated carbon

3. Biochar (several sources to reflect different source material and manufacture option of high O2 or not)
4. Wood chip or bark mulch
5. Zeolite
6. Perlite
7. Water treatment residuals
8. 60% sand/ 40% compost default bioretention soil mix (BSM)
9. High performance bioretention soil mix (HPBSM)

- Reporting: A data summary report that summarizes key research findings, data, and implications for treatment system performance. The report will be accompanied by a spreadsheet of results.

7.1 Study boundaries

This study uses laboratory-based experiments that will be performed at the University of Washington Tacoma, Center for Urban Waters. The media being tested are widely used in Washington State.

7.2 Field data collection

This study uses laboratory-based experiments that will be performed at the University of Washington Tacoma, Center for Urban Waters. No sampling of ambient environment will occur, although the natural soils will be sourced from a local municipal partner. The preferred natural soil will meet Ecology's soil suitability criteria. These soil samples will be extracted and analyzed for background 6PPDQ concentrations or presence prior to lab studies.

7.2.1 Sampling locations and frequency

Laboratory experiments will be performed between January 2023 (preliminary screening studies) and June 2023. Soil and treatment media samples will be collected from different locations (the final soils and media will be agreed upon with Ecology). Collected sorbent media will be subjected to pretreatment (e.g., autoclave) and characterization (e.g., measurement of TOC) before use. 6PPDQ methanolic stock solutions will be prepared following the method described in Appendix A. The sorption experiment will follow OECD 106 and Tang et al. (2023) with minor alterations. Briefly, sorption experiments will be conducted with different sorbent materials, different sorbent masses, and different initial 6PPDQ concentrations. The initial concentration of 6PPDQ will be achieved by adding different volumes of the stock solution (in methanol; final methanol composition should <0.1% v/v; OECD 106) into the pH-adjusted buffer solution (sodium phosphate buffer). The solution will be transferred into precleaned glass centrifuge tubes that contain sterilized sorbent for sorption experiments. The tubes will be equilibrated under room temperature (25 °C) on the shaker. Samples will be either taken at

time points spaced over the duration of the experiment (i.e., at 4 h to 7 d intervals) for the sorption kinetic study or at the end of the sorption (e.g., at 3 d) for the batch equilibrium study. The initial concentration of 6PPDQ, as well as sorbent mass load and sampling time points will be modified as needed based on observations during initial experiments (e.g., if 6PPDQ concentration changes are observed to occur rapidly). From a given experimental system, both water and soil samples will be collected for analysis with experimental triplicates used to capture experimental variability (i.e., triplicate tubes will be prepared for each time/sorbent/initial concentration condition). Tubes will be centrifuged (e.g., 3000 rpm, 20 min) to separate the solid and aqueous phases, and the supernatant will be collected for LC-MS/MS analysis. To evaluate the sorption kinetics and constants, 2-3 natural materials and 6-9 treatment media types will be acquired and tested. For each tested material, at least 3-7 time points will be examined with triplicate replication to determine the sorption kinetics. Desorption studies will proceed immediately upon conclusion of sorption studies, where the aqueous media is replaced with clean water and a new time series of aqueous samples and mass balances is begun.

Approximately 200-300 preliminary screening and final system samples are anticipated for 6PPDQ analysis by mass spectrometry-based methods.

7.2.2 Laboratory analytes to be measured

Laboratory analysis of water samples collected during 6PPDQ sorption experiments will consist of triplicate experimental replicates and method blanks. Extractions of these samples will be performed according to CUW SOP “Extraction and Analysis of 6PPD-Quinone in Water (Appendix A). The sorbent materials will be extracted following OECD 106 and Tang et al. Both water and sorbent samples will be analyzed to quantify 6PPDQ at the UWT CUW laboratory utilizing established LC-MS/MS techniques (Appendix A). The measurements of soils or treatment media properties will occur prior to sorption experiments, including pH, clay content and soil texture (as appropriate based on experimental conditions).

7.4 Assumptions of study design

A key assumption of the study design is that laboratory studies and systems are sufficiently representative of 6PPDQ sorption to different sorbent materials (natural soils or treatment medias) in terms of their sorption dynamics and constants. The scientific literature and related standardization efforts (e.g., use of OECD Method 106) supports this assumption.

7.5 Possible challenges and contingencies

The main focus of this project is to perform a suite of laboratory experiments to evaluate the partitioning dynamics and kinetics of 6PPDQ to soils and engineered sorbents. The use of laboratory studies limits challenges related to access or other environmental factors.

7.5.1 Logistical problems

A logistical problem that may impact the study is that 6PPDQ is potentially unstable (prone to oxidize/polymerize) in some of the experimental systems, which may strongly impact outcomes for equilibrium and kinetic observations. Because so little is known about 6PPDQ stability currently, we also reasonably anticipate potential instabilities in certain systems or certain solution conditions due to reactive mineral phases, dissolved organic carbon, or sorbent compositions. To address this, samples will be extracted and analyzed as soon as possible after generation (see Table 5 and Section 8.3). Mass balance analysis of experimental systems will be used to evaluate primary instability and basic fate assessments derived from literature on quinone stabilization or performed experimentally will be used to guide sample processing, extraction, and handling.

Another logistical problem is the low solubility of 6PPDQ in water (~35 ug/L in our studies). To address this, 6PPDQ stock solution will be prepared in methanol to ensure desired initial concentrations are achieved.

7.5.2 Practical constraints

The number and type of soils and engineered sorbents that can be evaluated may be limited by the availability of these materials and the consistency/similarity of some of the sorption outcomes expected for these systems. While we are designing these studies for high throughput and scalability, it is not currently known how many discrete systems can be evaluated within the project period. This will be mitigated to the extent possible by ordering supplies, setting up experiments, and training personnel as early within the project period as possible.

7.5.3 Schedule limitations

The logistical and practical constraints listed in sections 7.5.1 and 7.5.2 may impact the proposed study schedule if additional time is required to obtain preliminary screening data or replication data for these studies. Given the tight timeframe, the project success is also contingent upon possible delays in ordering supplies and instrument performance. The continued strong performance of our LC-MS/MS instrument throughout the project period is necessary for successful completion within the specified timeframes.

Given the short timeframes, the time required for QAPP review/approval may impact schedule. If additional funding from Ecology, that extends past June 30, 2023 becomes available, the project is anticipated to be extended to complete work under this QAPP.

8.0 Field Procedures

While no samples are being collected in the field, the details in this section describe measurement and sampling procedures for laboratory sorption and kinetic studies.

8.2 Measurement and sampling procedures

General procedures for laboratory experiment sampling are described below. Additional specific procedures for preparing sample collection equipment and materials, collecting water samples, handling samples before extraction and LC-MS/MS analysis, and sample/data analysis are included in the SOP entitled “Extraction and Analysis of 6PPD-Quinone in Water” (Appendix A).

8.3 Containers, preservation methods, holding times

Studies and samples will be collected in 10 mL pre-cleaned glass tubes (5 mL headspace). Experimental systems will be replicated in triplicate to the extent possible and independently analyzed. Water samples will be held at 4 °C until extraction and extracted within 24 h of sample generation. For kinetic studies, water samples will be typically extracted immediately, and solid phase extraction (SPE) extracts stored at -20 °C until batch analysis by LC-MS/MS. Soil and sorbent media (solids) will be held at -20 °C until extraction and extracted within 30 days of sample generation. Following processing by SPE, samples will be eluted from SPE using 100% methanol (see Appendix A) and are typically analyzed immediately by LC-MS/MS or stored at -20 °C until batch analysis and further sample archiving. Sample containers, preservation, and holding times are outlined in Table 5. Complete procedures for sample preparation are described in the CUW SOP entitled “Extraction and Analysis of 6PPD-Quinone in Water” (Appendix A).

Table 5. Sample containers, preservation, and holding times until processing or analysis.

Parameter	Matrix	Minimum Quantity Required	Container	Preservative	Holding Time
6PPDQ	Water	1 mL	Glass tube	n/a	<24 h, 4 ºC
6PPDQ	Soil/Sorbent	100 mg	Glass tube	n/a	<30 d, -20 ºC

8.4 Equipment decontamination

Disposable lab gloves will be worn during sample collection. Water sample bottles, caps, and associated laboratory equipment for experimentation and sampling are cleaned in the laboratory prior to the laboratory study. The cleaning procedure will include rinsing with

ultrapure water and organic solvent (methanol, ethyl acetate), followed by baking, to reduce laboratory and carryover contamination.

8.5 Sample ID

All sample containers and extracts will be labeled with a unique sample identifier that is recorded in relevant laboratory notebooks. Sample labels will include the date of collection, unique sample name/identifier that indicates both the sorbent/soil type and any other experimental variable under consideration, replicate number, time of collection (e.g., 0 h to indicate collection at the start of the experiment), and initials of laboratory personnel.

Chemical stocks and standards will be labeled with the name of the chemical, the chemical concentration, the date of preparation, and initials of the laboratory personnel.

8.6 Chain of custody

All samples will be generated and analyzed at CUW, so no chain of custody is necessary.

9.0 Laboratory Procedures

9.1 Lab procedures table

Table 6. Measurement methods for 6PPDQ analysis by LC-MS/MS

Analyte	6PPDQ
Sample Matrix and Site	Water samples or soil/sorbent samples (laboratory generated)
Number of Lab QC samples	See Table 7
Sample Preparation Method	Solid phase extraction (Appendix A), ASE (methods to be developed)
Analytical Method	LC-MS/MS (Appendix A)
Detection or Reporting Limit	MDL = 0.05 ng/mL in vial, MQL = 0.16 ng/mL in vial (1 mL volume for LC-MS/MS analysis)

9.2 Sample preparation method(s)

Depending on the system concentration of 6PPDQ, water samples for LC-MS/MS analysis will be either analyzed directly or prepared and solid phase extracted by methods outlined in the CUW SOP "Extraction and Analysis of 6PPD-Quinone in Water" (Appendix A). Briefly:

- Samples are spiked with a known mass of an isotopically labeled internal standard (6PPD-Q internal standard).
- Samples are passed through a pre-conditioned solid phase extraction cartridge for clean-up and to capture/concentrate 6PPDQ;
- Solvent is passed through the SPE cartridge to elute 6PPDQ;
- Solvent is blown down to a known volume and placed in autosampler vials for analysis.

9.3 Special method requirements

Not applicable.

9.4 Laboratories accredited for methods

There are no labs accredited by the Washington State Department of Ecology for 6PPDQ analysis from water or sediment matrices. Analyses of 6PPDQ will be performed at the UWT CUW laboratory. CUW submitted method accreditation materials for 6PPDQ to Ecology in June 2021. Since that period, a laboratory accreditation waiver has been obtained from Ecology for use of LC-MS/MS analyses for 6PPDQ quantification relative to the detection and reporting limits. Protocols and workflows are described in the CUW Laboratory SOP for sample collection,

sample processing, sample analysis, and data handling, data analysis, and data management. This SOP (Appendix A) is available upon request from the authors.

10.0 Quality Control Procedures

The quality control procedures that will help identify problems or issues associated with data collection and data analysis while the project is underway will include reviewing laboratory notes prior to completion of each experiment, following the laboratory procedures outlined in Section 9.0, following the QC procedures outlined in Section 6.0, and weekly staff meetings to review data outputs, analyses, progress on tasks, and logistics for upcoming tasks.

10.1 Table of field and laboratory quality control

Table 7. Quality control samples, types, and frequency for LC-MS/MS analysis.

Laboratory/Experiment Spikes	Minimum 2 LCS per analytical batch
Laboratory/Experiment (Method) Blanks	Minimum 1 Method Blank per analytical batch; 1 Method Blank per experimental condition
Laboratory/Experiment Replicates	Minimum 3 experimental replicates per sample type, as permitted by sample volume/mass availability
Calibration standards	CCV sample (CAL-4) is analyzed every 20-40 samples and at beginning/end of analytical run.
Instrument blanks	Methanol blank is analyzed every 20-40 samples through an analytical run

10.2 Corrective action processes

Project personnel will review laboratory and sample documentation to ensure that processes were performed according to the QA procedures, and to check for deficiencies and nonconformances. Deficiencies are unauthorized deviations from procedures documented in the QAPP. Nonconformances are deficiencies that affect quality and render the data unacceptable or indeterminate. Examples include:

- Deficiencies
  Conducting laboratory Quality Control sampling at a rate less than described in the QAPP.
- Nonconformance
  Failure to analyze samples within 24 h

Deficiencies or nonconformances are reported to the Project Manager, and corrective actions are applied (when possible) in a timely manner. Laboratory sample results found outside of warning limits (see MQOs) will be flagged for further evaluation and re-analyzed. The Project Manager is responsible for implementing and tracking corrective action procedures based on review findings. Records of corrective actions are maintained by the laboratory QA manager

(analytical chemistry), or the Project Manager (laboratory experiments). Experimental deficiencies and nonconformances are documented in sample logbooks.

11.0 Data Management Procedures

11.1 Data recording and reporting requirements

All electronic data, including documents, analytical output, statistical analysis, reports, etc. will be stored on project computers at the CUW that are backed up by a commercial cloud-based system that maintains continuously updated copies of all materials. A Microsoft Excel-based electronic record of all sampling events, stored samples, and associated data will be maintained for the project, and will be stored on CUW project computers. Data entry errors will be detected by comparison of laboratory notebook records and electronic data records.

Two types of documentation will be managed: (1) laboratory records and (2) QAPP revision documentation.

11.2 Laboratory data package requirements

The CUW laboratory will provide a data package to the Project Manager, or designee, and will be available to Ecology. The data package will report the outcomes of test results clearly and accurately. The test report will include the information necessary for interpretation and validation of data and will include the following:

- Report title
- Name and address of laboratory
- Cover narrative
- Study name
- Study and sample description
- Description of sample processing and sample analysis
- Analytical methods and results
- Results of all QA/QC samples
- An explanation of any failed QA/QC or non-standard conditions that may have affected data quality or study outcomes, including corrective actions and plan to prevent loss of quality

11.3 Electronic transfer requirements

All final results and data outputs will be entered into Microsoft Excel spreadsheets, presented as supporting information in the final report or related scientific manuscripts, and archived for at least 5 years.

11.4 Data upload procedures

Data from this study is not suitable for Ecology's database designed for water quality of receiving waters. In both publications and presentations, any data that have been generated in this study using non-accredited methods within this project will contain a disclaimer statement.

12.0 Audits and Reports

12.1 Audits

No audits are planned for these studies. All samples for this project will be collected following SOPs and guidelines described herein. All laboratory experiments and analyses of related samples will proceed pending approval of the QAPP.

12.3 Frequency and distribution of reports

Reports will include, per the project agreement, a QAPP, one progress report, and a final report including spreadsheet of data. Other outputs will include peer-reviewed manuscripts on the results of the study, although drafts of these are not expected to be completed within the project period. Reports will be submitted by email.

The progress report will summarize experimental progress, present and discuss findings, and describe all activities performed within the project period. The final report will summarize the background and goals of the project, the experimental design, data collection and analysis methods, data quality assessment results, and final results. It will provide conclusions and recommendations for follow-up activities, as appropriate. We will distribute the report for peer review and comment to Ecology before being finalized.

12.4 Responsibility for reports

Reports will be prepared by Edward Kolodziej, Ximin Hu, and Katherine Peter with contributions from the entire project team.

13.0 Data Verification

Data verification is a systematic process for evaluating performance and compliance of a set of data to ascertain its completeness, correctness, and consistency using the methods and criteria defined in the QAPP. All data obtained from laboratory measurements will be reviewed and verified. Verifying the data quality will help detect inaccuracies, characterize uncertainties, and identify other potential deficiencies. Only data that meet appropriate quality objectives and quality control procedures will be considered acceptable and used in the study. The validation process will involve assembling and comparing the raw data and QC sample results to determine if project MQOs have been met.

13.1 Field data verification, requirements, and responsibilities

No field data are collected during this project. For laboratory experiments, the Project Manager will review all records associated with the QC procedures described in Section 10 for evidence of proper procedures for sample handling, record keeping, and collection of blanks.

13.2 Laboratory data verification

The lab data verification procedures will involve assessing:

- Proper calibration and tuning of analytical instrumentation prior to use;
- Sample validity (e.g., sampling method and SOP were followed, no substantial difficulties were encountered collecting samples, correct sample handling and identification, suitable agreement (<20%) across replicate systems);
- Laboratory QA/QC (i.e., did the lab meet the MQOs?)

The project manager will conduct a compliance screening by evaluating sample holding times, evidence of blank contamination, precision (replicate analyses, reference standard analyses, tune reports), bias, and sensitivity (instrument resolving power, blank analyses, method detection limit). Instrument screening (initial calibration, continuing calibration, tuning, sensitivity, and degradation) will be performed as part of regular laboratory operations.

13.3 Validation requirements, if necessary

Not applicable.

14.0 Data Quality (Usability) Assessment

14.1 Process for determining project objectives were met

To evaluate whether the project outcomes have met the original objectives, the project manager will assess if the data were collected consistent with the study design (with no reason to question the study design assumptions), study methods, and study procedures described in the final approved QAPP, and if enough of the data (>90%) are deemed usable after verification (i.e., all relevant MQOs are met). The name of the data files for which LC-MS/MS data are rejected will marked with "_Rejected" at the end of the filename, and the rejection reason will be noted in the project laboratory notebook.

14.2 Treatment of non-detects

In the context of LC-MS/MS data, non-detects are defined as the lack of a peak with S/N ratio of <3, or below the linear range of the calibration curve, at the observed retention time for an analytical standard of 6PPDQ. Non-detects will be reported as ND in processed data outputs and in the data spreadsheet.

14.3 Data analysis and presentation methods

Data acquisition is performed in Agilent MassHunter Acquisition Version B.05.01, Build 5.01.5125.3. Data analysis is performed in Agilent MassHunter Quantitative Analysis software. Additional details are provided in the SOP "Extraction and Analysis of 6PPD-Quinone in Water" (Appendix A).

14.4 Sampling design evaluation

The study and sampling design is anticipated to provide sufficient statistical power to draw the desired conclusions. Use of experimental triplicates (along with the analysis of both laboratory and analytical blanks) will provide the ability to ensure statistical relevance of the datasets. Collection and comparison of quantitative data from multiple similar systems containing different soils or sorbents, across time, will provide the statistical power to account for variability in performance outcomes for these soils and sorbents.

14.5 Documentation of assessment

The data usability assessment and the decision to use the data in the analysis will be documented in the data report by reporting relevant parameters for the MQOs.

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16.0 Appendices

Appendix A. Extraction and Analysis of 6PPD-Quinone in Water

Appendix B. Glossaries, Acronyms, and Abbreviations

Appendix A. SOP: Extraction and Analysis of 6PPD-Quinone in Water

Available on request from the authors, University of Washington Tacoma,
Center for Urban Waters

Appendix B. Glossaries, Acronyms, and Abbreviations

Glossary of General Terms

Accurate mass: Experimentally determined mass of an ion of known charge (see also *m/z*). Can be used to determine elemental composition to within limits defined by both the accuracy and precision of the instrument (Murray et al., 2013).

Ambient: Background or away from point sources of contamination. Surrounding environmental condition.

Anthropogenic: Human-caused.

Baseflow: The component of total streamflow that originates from direct groundwater discharges to a stream.

Clean Water Act: A federal act passed in 1972 that contains provisions to restore and maintain the quality of the nation's waters. Section 303(d) of the Clean Water Act establishes the TMDL program.

Conductivity: A measure of water's ability to conduct an electrical current. Conductivity is related to the concentration and charge of dissolved ions in water.

Critical condition: When the physical, chemical, and biological characteristics of the receiving water environment interact with the effluent to produce the greatest potential adverse impact on aquatic biota and existing or designated water uses. For steady-state discharges to riverine systems, the critical condition may be assumed to be equal to the 7Q10 flow event unless determined otherwise by the department.

Designated uses: Those uses specified in Chapter 173-201A WAC (Water Quality Standards for Surface Waters of the State of Washington) for each water body or segment, regardless of whether or not the uses are currently attained.

Dilution factor: The relative proportion of effluent to stream (receiving water) flows occurring at the edge of a mixing zone during critical discharge conditions as authorized in accordance with the state's mixing zone regulations at WAC 173-201A-100.
http://apps.leg.wa.gov/WAC/default.aspx?cite=173-201A-020

Effluent: An outflowing of water from a natural body of water or from a human-made structure. For example, the treated outflow from a wastewater treatment plant.

Exact mass: Calcuated mass of an ion or molecule with specified isotopic compositions (see also **accurate mass**). Exact mass of an ion or molecule is calculated as a monoisotopic mass using the most abundant isotope of each element (Murray et al., 2013).

Existing uses: Those uses actually attained in fresh and marine waters on or after November 28, 1975, whether or not they are designated uses. Introduced species that are not native to Washington, and put-and-take fisheries comprised of non-self-replicating introduced native species, do not need to receive full support as an existing use.

Geometric mean: A mathematical expression of the central tendency (an average) of multiple sample values. A geometric mean, unlike an arithmetic mean, tends to dampen the effect of very high or low values, which might bias the mean if a straight average (arithmetic mean) were calculated. This is helpful when analyzing bacteria concentrations, because levels may vary anywhere from 10 to 10,000 fold over a given period. The calculation is performed by either: (1) taking the nth root of a product of n factors, or (2) taking the antilogarithm of the arithmetic mean of the logarithms of the individual values.

Liquid chromatography-mass spectrometry (LC-MS): Technique by which a mixture of analytes is separated into individual components by liquid chromatography (typically high-performance liquid chromatography), followed by detection with a mass spectrometer (Murray et al., 2013).

Load allocation: The portion of a receiving water's loading capacity attributed to one or more of its existing or future sources of nonpoint pollution or to natural background sources.

Loading capacity: The greatest amount of a substance that a water body can receive and still meet water quality standards.

Municipal separate storm sewer systems (MS4): A conveyance or system of conveyances (including roads with drainage systems, municipal streets, catch basins, curbs, gutters, ditches, manmade channels, or storm drains): (1) owned or operated by a state, city, town, borough, county, parish, district, association, or other public body having jurisdiction over disposal of wastes, stormwater, or other wastes and (2) designed or used for collecting or conveying stormwater; (3) which is not a combined sewer; and (4) which is not part of a Publicly Owned Treatment Works (POTW) as defined in the Code of Federal Regulations at 40 CFR 122.2.

***m/z*:** Abbreviation representing the dimensionless quantity formed by dividing the ratio of the mass of an ion to the unified atomic mass unit, by its charge number; Mass-to-charge (m/z) is a depreciated term. Mass-to-charge ratio often represents the horizontal axis in a plot of a mass spectrum (Murray et al., 2013). Mass here refers to accurate mass.

Mass peak: (or peak) Localized region of relatively intense detector response in a mass spectrum when ions of a specified *m/z* are detected. Although mass peaks are often associated with particular ions, the terms *peak* and *ion* should not be used interchangeably (Murray et al., 2013).

Mass spectrometry (MS): Study of matter through the formation of gas-phase ions that are characterized using mass spectrometers by their mass, charge, structure and/or physico-chemical properties. Mass spectrometer is the instrument that measures the *m/z* values and the abundances of gas-phase *ions* (Murray et al., 2013).

Mass spectrometry/mass spectrometry (QQQ, MS/MS; MS^2) or tandem mass spectrometry: Acquisition and study of the spectra of the product ions or precursor ions of m/z selected ions, or of precursor ions of a selected neutral mass loss (Murray et al., 2013).

Non target chemical feature: Ions (with grouped adducts and isotopologues) generated by LC-HRMS that are defined by both their *m/z* and chromatographic retention time.

Non target liquid chromatography - high resolution mass spectrometry (LC-HRMS): Mixtures of analytes must first be separated by liquid chromatography prior to analysis by mass spectrometry. Operation of a high resolution mass spectrometer in which the abundances of ions for all *m/z* values in the entire mass spectrum are recorded. Small molecule identification occurs both through the accurate (exact) mass of the precursor ions generated as well as identification by tandem mass spectrometry.

Nonpoint source: Pollution that enters any waters of the state from any dispersed land-based or water-based activities, including but not limited to atmospheric deposition, surface-water runoff from agricultural lands, urban areas, or forest lands, subsurface or underground sources, or discharges from boats or marine vessels not otherwise regulated under the NPDES program. Generally, any unconfined and diffuse source of contamination. Legally, any source of water pollution that does not meet the legal definition of "point source" in section 502(14) of the Clean Water Act.

pH: A measure of the acidity or alkalinity of water. A low pH value (0 to 7) indicates that an acidic condition is present, while a high pH (7 to 14) indicates a basic or alkaline condition. A pH of 7 is considered to be neutral. Since the pH scale is logarithmic, a water sample with a pH of 8 is ten times more basic than one with a pH of 7.

Point source: Source of pollution that discharges at a specific location from pipes, outfalls, and conveyance channels to a surface water. Examples of point source discharges include municipal wastewater treatment plants, municipal stormwater systems, industrial waste treatment facilities, and construction sites where more than 5 acres of land have been cleared.

Pollution: Contamination or other alteration of the physical, chemical, or biological properties of any waters of the state. This includes change in temperature, taste, color, turbidity, or odor of the waters. It also includes discharge of any liquid, gaseous, solid, radioactive, or other substance into any waters of the state. This definition assumes that these changes will, or are likely to, create a nuisance or render such waters harmful, detrimental, or injurious to (1) public health, safety, or welfare, or (2) domestic, commercial, industrial, agricultural, recreational, or other legitimate beneficial uses, or (3) livestock, wild animals, birds, fish, or other aquatic life.

Quadrupole time-of-flight (QTOF): Hybrid mass spectrometer consisting of a transmission quadrupole mass spectrometer coupled to an orthogonal acceleration time-of-flights mass spectrometer (Murray et al., 2013).

Reference ions or masses: Stable ion whose structure or elemental formula is known with certainty. These ion are formed by direct ionization of a molecule with a known structure or elemental formula, and are used to verify by comparison the structure or thermochemistry of an unknown ion or to calibrate the *m/z* scale of the mass spectrometer (Murray et al., 2013).

Salmonid: Fish that belong to the family *Salmonidae*. Species of salmon, trout, or char.

Sediment: Soil and organic matter that is covered with water (for example, river or lake bottom).

Stormwater: The portion of precipitation that does not naturally percolate into the ground or evaporate but instead runs off roads, pavement, and roofs during rainfall or snow melt. Stormwater can also come from hard or saturated grass surfaces such as lawns, pastures, playfields, and from gravel roads and parking lots.

Streamflow: Discharge of water in a surface stream (river or creek).

Surface waters of the state: Lakes, rivers, ponds, streams, inland waters, salt waters, wetlands and all other surface waters and water courses within the jurisdiction of Washington State.

Total suspended solids (TSS): Portion of solids retained by a filter.

Turbidity: A measure of water clarity. High levels of turbidity can have a negative impact on aquatic life.

Watershed: A drainage area or basin in which all land and water areas drain or flow toward a

90th percentile: An estimated portion of a sample population based on a statistical determination of distribution characteristics. The 90th percentile value is a statistically derived estimate of the division between 90% of samples, which should be less than the value, and 10% of samples, which are expected to exceed the value.

Acronyms and Abbreviations

BMP	Best management practice
CUW	Center for Urban Waters
DO	Dissolved oxygen
DOC	Dissolved organic carbon
e.g.	For example
Ecology	Washington State Department of Ecology
EIM	Environmental Information Management database
EPA	U.S. Environmental Protection Agency
et al.	And others
FC	Fecal coliform
GIS	Geographic Information System software
GPS	Global Positioning System
HRMS	High-resolution mass spectrometry
i.e.	In other words
IDL	Instrument detection limit
IQL	Instrument quantification limit
LC	Liquid chomatography
MDL	Method detection limit (see Quality assurance glossary below)
MQL	Method quantification limit
MQO	Measurement quality objective
MS	Mass spectrometry
MS/MS	Tandem mass spectrometry
NPDES	National Pollutant Discharge Elimination System
QA	Quality assurance
QC	Quality control
RPD	Relative percent difference
RSD	Relative standard deviation
SOP	Standard operating procedures
SRM	Standard reference materials

TMDL	Total Maximum Daily Load
TOC	Total organic carbon
TSS	Total suspended solids
URMS	Urban runoff mortality syndrome
USGS	United States Geological Survey
UWT	University of Washington Tacoma
WAC	Washington Administrative Code
WRIA	Water Resource Inventory Area
WQA	Water Quality Assessment
WSU-P	Washington State University-Puyallup

Units of Measurement

°C	degrees centigrade
cfs	cubic feet per second
cfu	colony forming units
cms	cubic meters per second, a unit of flow
dw	dry weight
ft	feet
g	gram, a unit of mass
kcfs	1000 cubic feet per second
kg	kilograms, a unit of mass equal to 1,000 grams
kg/d	kilograms per day
km	kilometer, a unit of length equal to 1,000 meters
L/s	liters per second (0.03531 cubic foot per second)
m	meter
mm	millimeter
mg	milligram
mgd	million gallons per day
mg/d	milligrams per day
mg/kg	milligrams per kilogram (parts per million)
mg/L	milligrams per liter (parts per million)

mg/L/hr	milligrams per liter per hour
mL	milliliter
mmol	millimole or one-thousandth of a mole
mole	an International System of Units (IS) unit of matter
ng/g	nanograms per gram (parts per billion)
ng/kg	nanograms per kilogram (parts per trillion)
ng/L	nanograms per liter (parts per trillion)
NTU	nephelometric turbidity units
pg/g	picograms per gram (parts per trillion)
pg/L	picograms per liter (parts per quadrillion)
psu	practical salinity units
s.u.	standard units
µg/g	micrograms per gram (parts per million)
µg/kg	micrograms per kilogram (parts per billion)
µg/L	micrograms per liter (parts per billion)
µm	micrometer
µM	micromolar (a chemistry unit)
µmhos/cm	micromhos per centimeter
µS/cm	microsiemens per centimeter, a unit of conductivity
ww	wet weight

Quality Assurance Glossary

Accreditation: A certification process for laboratories, designed to evaluate and document a lab's ability to perform analytical methods and produce acceptable data (Kammin, 2010). For Ecology, it is defined according to WAC 173-50-040: "Formal recognition by [Ecology] that an environmental laboratory is capable of producing accurate and defensible analytical data."

Accuracy: The degree to which a measured value agrees with the true value of the measured property. USEPA recommends that this term not be used, and that the terms *precision* and *bias* be used to convey the information associated with the term *accuracy* (USEPA, 2014).

Analyte: An element, ion, compound, or chemical moiety (pH, alkalinity) which is to be determined. The definition can be expanded to include organisms, e.g., fecal coliform, Klebsiella (Kammin, 2010).

Bias: Discrepancy between the expected value of an estimator and the population parameter being estimated (Gilbert, 1987; USEPA, 2014).

Blank: A synthetic sample, free of the analyte(s) of interest. For example, in water analysis, pure water is used for the blank. In chemical analysis, a blank is used to estimate the analytical response to all factors other than the analyte in the sample. In general, blanks are used to assess possible contamination or inadvertent introduction of analyte during various stages of the sampling and analytical process (USGS, 1998).

Calibration: The process of establishing the relationship between the response of a measurement system and the concentration of the parameter being measured (Ecology, 2004).

Check standard: A substance or reference material obtained from a source independent from the source of the calibration standard; used to assess bias for an analytical method. This is an obsolete term, and its use is highly discouraged. See Calibration Verification Standards, Lab Control Samples (LCS), Certified Reference Materials (CRM), and/or spiked blanks. These are all check standards but should be referred to by their actual designator, e.g., CRM, LCS (Kammin, 2010; Ecology, 2004).

Comparability: The degree to which different methods, data sets and/or decisions agree or can be represented as similar; a data quality indicator (USEPA, 2014; USEPA, 2020).

Completeness: The amount of valid data obtained from a project compared to the planned amount. Usually expressed as a percentage. A data quality indicator (USEPA, 2014; USEPA 2020).

Continuing Calibration Verification Standard (CCV): A quality control (QC) sample analyzed with samples to check for acceptable bias in the measurement system. The CCV is usually a midpoint calibration standard that is re-run at an established frequency during the course of an analytical run (Kammin, 2010).

Control chart: A graphical representation of quality control results demonstrating the performance of an aspect of a measurement system (Kammin, 2010; Ecology 2004).

Control limits: Statistical warning and action limits calculated based on control charts. Warning limits are generally set at +/- 2 standard deviations from the mean, action limits at +/- 3 standard deviations from the mean (Kammin, 2010).

Data integrity: A qualitative DQI that evaluates the extent to which a data set contains data that is misrepresented, falsified, or deliberately misleading (Kammin, 2010).

Data quality indicators (DQI): Commonly used measures of acceptability for environmental data. The principal DQIs are precision, bias, representativeness, comparability, completeness, sensitivity, and integrity (USEPA, 2006).

Data quality objectives (DQO): Qualitative and quantitative statements derived from systematic planning processes that clarify study objectives, define the appropriate type of data, and specify tolerable levels of potential decision errors that will be used as the basis for establishing the quality and quantity of data needed to support decisions (USEPA, 2006).

Data set: A grouping of samples organized by date, time, analyte, etc. (Kammin, 2010).

Data validation: The process of determining that the data satisfy the requirements as defined by the data user (USEPA, 2020). There are various levels of data validation (USEPA, 2009).

Data verification: Examination of a data set for errors or omissions, and assessment of the Data Quality Indicators related to that data set for compliance with acceptance criteria (MQOs). Verification is a detailed quality review of a data set (Ecology, 2004).

Detection limit (limit of detection)**:** The concentration or amount of an analyte which can be determined to a specified level of certainty to be greater than zero (Ecology, 2004).

Duplicate samples: Two samples taken from and representative of the same population, and carried through and steps of the sampling and analytical procedures in an identical manner. Duplicate samples are used to assess variability of all method activities including sampling and analysis (USEPA, 2014).

Field blank: A blank used to obtain information on contamination introduced during sample collection, storage, and transport (Ecology, 2004).

Initial Calibration Verification Standard (ICV): A QC sample prepared independently of calibration standards and analyzed along with the samples to check for acceptable bias in the measurement system. The ICV is analyzed prior to the analysis of any samples (Kammin, 2010).

Laboratory Control Sample (LCS)/LCS duplicate: A sample of known composition prepared using contaminant-free water or an inert solid that is spiked with analytes of interest at the midpoint of the calibration curve or at the level of concern. It is prepared and analyzed in the same batch of regular samples using the same sample preparation method, reagents, and

analytical methods employed for regular samples. Monitors a lab's performance for bias and precision (USEPA, 2014).

Matrix spike/Matrix spike duplicate: A QC sample prepared by adding a known amount of the target analyte(s) to an aliquot of a sample to check for bias and precision errors due to interference or matrix effects (Ecology, 2004).

Measurement Quality Objectives (MQOs)**:** Performance or acceptance criteria for individual data quality indicators, usually including precision, bias, sensitivity, completeness, comparability, and representativeness (USEPA, 2006).

Measurement result: A value obtained by performing the procedure described in a method (Ecology, 2004).

Method: A formalized group of procedures and techniques for performing an activity (e.g., sampling, chemical analysis, data analysis), systematically presented in the order in which they are to be executed (USEPA, 2001).

Method blank: A blank prepared to represent the sample matrix, prepared and analyzed with a batch of samples. A method blank will contain all reagents used in the preparation of a sample, and the same preparation process is used for the method blank and samples (Ecology, 2004; Kammin, 2010).

Method Detection Limit (MDL): The minimum measured concentration of a substance that can be reported with 99% confidence that the measured concentration is distinguishable from method blank results (USEPA, 2016). MDL is a measure of the capability of an analytical method of distinguished samples that do not contain a specific analyte from a sample that contains a low concentration of the analyte (USEPA, 2020).

Minimum level: Either the sample concentration equivalent to the lowest calibration point in a method or a multiple of the method detection limit (MDL), whichever is higher. For the purposes of NPDES compliance monitoring, EPA considers the following terms to be synonymous: "quantitation limit," "reporting limit," and "minimum level" (40 CFR 136).

Parameter: A specified characteristic of a population or sample. Also, an analyte or grouping of analytes. Benzene and nitrate + nitrite are all parameters (Kammin, 2010; Ecology, 2004).

Population: The hypothetical set of all possible observations of the type being investigated (Ecology, 2004).

Precision: The extent of random variability among replicate measurements of the same property; a data quality indicator (USGS, 1998).

Quality assurance (QA): A set of activities designed to establish and document the reliability and usability of measurement data (Kammin, 2010).

Quality Assurance Project Plan (QAPP): A document that describes the objectives of a project, and the processes and activities necessary to develop data that will support those objectives (Kammin, 2010; Ecology, 2004).

Quality control (QC): The routine application of measurement and statistical procedures to assess the accuracy of measurement data (Ecology, 2004).

Relative Percent Difference (RPD): RPD is commonly used to evaluate precision. The following formula is used:

$$RPD = [Abs(a-b)/((a + b)/2)] * 100\%$$

where "Abs()" is absolute value and a and b are results for the two replicate samples. RPD can be used only with 2 values. Percent Relative Standard Deviation is (%RSD) is used if there are results for more than 2 replicate samples (Ecology, 2004).

Relative Standard Deviation (RSD): A statistic used to evaluate precision in environmental analysis. It is determined in the following manner:

$$RSD = (100\% * s)/x$$

where s is the sample standard deviation and x is the mean of results from more than two replicate samples (Kammin, 2010).

Replicate samples: Two or more samples taken from the environment at the same time and place, using the same protocols. Replicates are used to estimate the random variability of the material sampled (USGS, 1998).

Reporting level: Unless specified otherwise by a regulatory authority or in a discharge permit, results for analytes that meet the identification criteria (i.e., rules for determining qualitative presence/absence of an analyte) are reported down to the concentration of the minimum level established by the laboratory through calibration of the instrument. EPA considers the terms "reporting limit," "quantitation limit," and "minimum level" to be synonymous (40 CFR 136).

Representativeness: The degree to which a sample reflects the population from which it is taken; a data quality indicator (USGS, 1998).

Sample (field): A portion of a population (environmental entity) that is measured and assumed to represent the entire population (USGS, 1998).

Sample (statistical): A finite part or subset of a statistical population (USEPA, 1992).

Sensitivity: In general, denotes the rate at which the analytical response (e.g., absorbance, volume, meter reading) varies with the concentration of the parameter being determined. In a specialized sense, it has the same meaning as the detection limit (Ecology, 2004).

Spiked blank: A specified amount of reagent blank fortified with a known mass of the target analyte(s); usually used to assess the recovery efficiency of the method (USEPA, 2014).

Spiked sample: A sample prepared by adding a known mass of target analyte(s) to a specified amount of matrix sample for which an independent estimate of target analyte(s) concentration is available. Spiked samples can be used to determine the effect of the matrix on a method's recovery efficiency (USEPA, 2014).

Split sample: A discrete sample subdivided into portions, usually duplicates (Kammin, 2010).

Standard Operating Procedure (SOP): A document which describes in detail a reproducible and repeatable organized activity (Kammin, 2010).

Surrogate: For environmental chemistry, a surrogate is a substance with properties similar to those of the target analyte(s). Surrogates are unlikely to be native to environmental samples. They are added to environmental samples for quality control purposes, to track extraction efficiency and/or measure analyte recovery. Deuterated organic compounds are examples of surrogates commonly used in organic compound analysis (Kammin, 2010).

Systematic planning: A step-wise process which develops a clear description of the goals and objectives of a project, and produces decisions on the type, quantity, and quality of data that will be needed to meet those goals and objectives. The DQO process is a specialized type of systematic planning (USEPA, 2006).

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Standard Operating Procedure
Extraction and Analysis of 6PPD-Quinone in Water

January 2023

University of Washington Tacoma (UWT) at the Center for Urban Waters (CUW)

Publication Information

The University of Washington Tacoma laboratories at the Center for Urban Waters develops Standard Operating Procedures (SOPs) to document practices related to sampling, field and laboratory analysis, and other aspects of the laboratory's technical operations.

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SOP Revision History

Reviser(s)	Date	Notes
Katherine Peter	1/20/2023	Original draft

1 Table of Contents

1.1 List of Figures

1.2 List of Tables

2 Purpose, Scope, and Application

This document is the University of Washington Tacoma (UWT) laboratories at the Center for Urban Waters (CUW) Standard Operating Procedure (SOP) for the quantitative analysis by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) of 6PPD-Quinone (6PPDQ) in water, using isotopic dilution. The analytical methods described herein are based on those detailed in Tian et al. 2022.[1] This SOP describes procedures for preparation of necessary stocks and calibration standards, for water sample collection, extraction, and analysis, and for data analysis.

This SOP is applicable to extraction and analysis of 6PPDQ in water. Other analytes and matrices may be added if they meet the minimum QC requirements as outlined in this document.

Analyte identifications are confirmed by retention time, a precursor ion, a product quantifier ion, at least 1 product qualifier ion, and the ratio between these two product ions.

The detection limits and quantitation levels in this method are usually dependent on the level of interferences rather than instrumental limitations. The method detection limits (MDLs) and lower limits of quantitation (LLOQs) are the levels at which the analytes can be determined in the absence of interferences.

3 Personnel Qualifications and Responsibilities

The analysis in this method is restricted to use by or under the supervision of analysts experienced in the use of liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) and the interpretation of chromatograms and mass spectra. Training in this procedure with experienced personnel and completion of training checklists specific to the UWT-CUW laboratories is recommended.

4 Definitions and Abbreviations

4.1 Units of weight and measure and their abbreviations

°C	degrees Celsius
µL	microliter
<	less than
>	greater than
%	percent
cm	centimeter
g	gram
h	hour

in	inch
L	liter
M	molecular ion
m	meter
mg	milligram
min	minute
mL	milliliter
mm	millimeter
m/z	mass to charge ratio
pg	picogram
ppb	part-per-billion
ppm	part-per-million
ppt	part-per-trillion
v/v	volume per unit volume
w/v	weight per unit volume

4.2 Definitions and acronyms (in alphabetical order)

Analyte	An element, ion, compound, or chemical moiety (pH, alkalinity) which is to be determined.
Analytical Batch	A group of samples that are analyzed within the same analytical worklist.
Bias	Discrepancy between the expected value of an estimator and the population parameter being estimated.
Blank	A synthetic sample, free of the analyte(s) of interest. For example, in water analysis, pure water is used for the blank. In chemical analysis, a blank is used to estimate the analytical response to all factors other than the analyte in the sample. In general, blanks are used to assess possible contamination or

	inadvertent introduction of analyte during various stages of the sampling and analytical process.
Blind to the Analyst (BTTA) Study	Study performed to demonstrate initial and on-going method performance.
Calibration	The process of establishing the relationship between the response of a measurement system and the concentration of the parameter being measured.
Continuing Calibration Verification Standard (CCV)	A quality control (QC) sample analyzed with samples to check for acceptable bias in the measurement system. The CCV is usually a midpoint calibration standard that is re-run at an established frequency during the course of an analytical run.
Control charts	Charts used to a graphic representation of continued laboratory performance, such as from ongoing precision and recovery evaluations.
Control limits	Statistical warning and action limits calculated based on control charts. Warning limits are generally set at +/- 2 standard deviations from the mean, action limits at +/- 3 standard deviations from the mean.
Corrective Action	Actions taken by the analyst if quality control samples do not meet defined acceptance criteria.
Data set	A grouping of samples organized by date, time, analyte, etc.
Duplicate samples (DUP)	Two samples taken from and representative of the same population, and carried through and steps of the sampling and analytical procedures in an identical manner. Duplicate samples are used to assess variability of all method activities including sampling and analysis.
Field blank	A field blank is deionized water that is taken into the field and exposed to the same conditions and equipment as field samples, and then extracted in the same manner as all samples. Field blanks may not be necessary or available for every study. Field blanks should be processed by the same method as used for samples, and they can be used to assess any contamination stemming from the sample collection effort.
Initial Calibration Verification Standard (ICV)	A QC sample prepared independently of calibration standards and analyzed along with the samples to check for acceptable bias in the measurement system. The ICV is analyzed prior to the analysis of any samples.
Initial Demonstrate of Capability (IDC) Study	Study performed to provide an initial demonstration of the ability to generate acceptable precision and recovery with the method. Also referred to as Initial Precision and Recovery (IPR).

Internal standard (ISTD)	Isotope-labeled standards that are spiked before the sample extract is analyzed. They are used in the instrument calibration, which involves a comparison of instrument responses of the target compound to responses of standards added to the sample/extract prior to analysis. The target compound is normalized to the internal standard. Internal standards are used in tandem with surrogates.
Isotope dilution internal standard (ID-ISTD)	A special case of internal standard that is added prior to extraction or sample preparation steps. They mirror sample prep and determinative loss of the target compound(s). ID-ISTDs are used without the need for surrogates, whereas internal standards are used in tandem with surrogates.
Isotope dilution quantitation	A means of determining the concentration of a naturally occurring (native) compound by reference to the same compound (or similar) compound in which one or more atoms has been isotopically enriched. The labeled analogs are spiked into each sample to allow identification and correction of the concentration of the native compound in the analytical process.
Laboratory Control Sample (LCS); Laboratory Control Sample Duplicate (LCSD)	A sample of known composition prepared using contaminant-free water or an inert solid that is spiked with analytes of interest at the midpoint of the calibration curve or at the level of concern. It is prepared and analyzed in the same batch of regular samples using the same sample preparation method, reagents, and analytical methods employed for regular samples. Spike percent recovery is determined for each laboratory control sample to evaluate ongoing precision and recovery. Here, deionized water is used to prepare the laboratory control sample(s).
LC	Liquid chromatography
Lower Limit of Quantitation (LLOQ)	The lowest point of quantitation, which, in most cases, is the lowest concentration in the calibration curve. Also referred to as the minimum level (ML), reporting limit (RL), or method quantitation limit (MQL).
MS/MS	Tandem mass spectrometry
Matrix Spike (MS), Matrix Spike Duplicate (MSD)	A QC sample prepared by adding a known amount of the target analyte(s) to an aliquot of a sample to check for bias due to interference or matrix effects. An appropriate sample is an environmental water sample with a similar or identical matrix to the field samples in the batch (e.g., road runoff, creek water). For each of these samples,

	the matrix spike percent recovery should be determined to evaluate bias due to potential matrix interference. Matrix spike duplicates are used to document the precision and bias of a method for a specific sample matrix.
Matrix Spike Duplicate (MSD)	An additional replicate of the matrix spike sample following the same sample preparation and analytical testing as the original sample. MSDs are used to document the precision and bias of a method for a specific sample matrix.
Measurement result	A value obtained by performing the procedure described in a method.
Methanol Blank	An aliquot of pure methanol, pipetted directly into an autosampler vial.
Method	A formalized group of procedures and techniques for performing an activity (e.g., sampling, chemical analysis, data analysis), systematically presented in the order in which they are to be executed.
Method Blank (MB)	A blank prepared to represent the sample matrix, prepared and analyzed with a batch of samples. The same preparation process is used for the method blank and samples. A method blank contains all reagents used in the preparation of a sample. Here, deionized water is used to prepare the method blank(s).
Method Detection Limit (MDL)	The Method Detection Limit (MDL) is the lowest concentration at which an analyte can be detected under routine operating conditions (see 40 CFR 136, Appendix B).
Ongoing Precision and Recovery (OPR)	Performance of the method, as evaluated by spike and recovery experiments conducted periodically.
Precision	The extent of random variability among replicate measurements of the same property; a data quality indicator
Preparation Batch	A group of samples that are extracted within the same 24-hour period
Quality assurance (QA)	A set of activities designed to establish and document the reliability and usability of measurement data.
Quality Assurance Project Plan (QAPP)	A document that describes the objectives of a project, and the processes and activities necessary to develop data that will support those objectives.
Quality control (QC)	The routine application of measurement and statistical procedures to assess the accuracy of measurement data.
Relative Percent Difference (RPD)	RPD is commonly used to evaluate precision. The following formula is used: RPD = [Abs(a-b)/((a + b)/2)] * 100%

	where "Abs()" is absolute value and a and b are results for the two replicate samples. RPD can be used only with 2 values. Percent Relative Standard Deviation is (%RSD) is used if there are results for more than 2 replicate samples.
Relative Standard Deviation (RSD)	A statistic used to evaluate precision in environmental analysis. It is determined in the following manner: RSD = (100% * s)/x where s is the sample standard deviation and x is the mean of results from more than two replicate samples.
Solid Phase Extraction (SPE)	A technique for sample clean-up and extracting contaminants of interest from water into a small volume of solvent for analysis.
Standard Operating Procedure (SOP)	A document which describes in detail a reproducible and repeatable organized activity.
Stock solution	A solution containing the analyte that is prepared using a reference material traceable to the Environmental Protection Agency (EPA), the National Institute of Standards and Technology (NIST), or a source that will attest to the purity and authenticity of the reference material.
Surrogate	Isotope-labeled standards that are spiked so they go through the whole sample preparation process with the samples. They provide information about the preparation and analytical process, such as extraction efficiency. Surrogates have properties similar to those of the target analyte(s).

5 Summary of Method

The general steps in this method are summarized below.

Stocks, spiking solutions, and calibration standards are prepared according to procedures described in Section 8.

Aqueous samples are collected and extracted by solid phase extraction (SPE) (250 mL sample aliquots, final extract volume 1 mL) following glassware cleaning procedures, sample collection methods, and extraction procedures described in Section 9.

Extracted samples are then analyzed by LC-MS/MS, as described in Section 10, with subsequent data analysis using Agilent software and Microsoft Excel, as described in Section 11. Quantitative analysis uses isotope dilution and a multipoint calibration of the target analytes.

Quality assurance and quality control (QA/QC) procedures are detailed in Section 12, with quality of the analysis ensured through reproducible calibration and testing of the calibration, extraction, and LC-MS/MS systems.

6 Interferences

Solvents, reagents, glassware, and other sample processing hardware may yield artifacts, elevated baselines, matrix enhancement or matrix suppression causing misinterpretation of chromatograms. Specific selection of reagents and purification of solvents by distillation in all-glass systems may be required. Where possible, reagents are cleaned by extraction or solvent rinse.

Proper cleaning of glassware is extremely important, because glassware may not only contaminate the samples but may also remove the analytes of interest by adsorption on the glass surface. Details regarding glassware cleaning procedures are described in Section 9.5.1.

All materials in the analysis must be demonstrated to be free from interferences by running method blanks. Details regarding quality assurance and quality control procedures are described in Section 12.

Interferences co-extracted from samples will vary considerably from source to source, depending on the diversity of the site being sampled. Interfering compounds may be present at concentrations several orders of magnitude higher than the analytes of interest. Because low concentrations are measured by this method, elimination of interferences is essential. The extraction steps given in Section 9 can be used to reduce or eliminate these interferences and thereby permit reliable determination of the analytes.

7 Safety

The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound must be treated as a potential health hazard. Accordingly, exposure to these chemicals must be reduced to the lowest possible level.

The analysts should be familiar with the location and proper use of the fume hoods, eye washes, safety showers, and fire extinguishers. The analysts must wear personal protective equipment at all times, including safety glasses.

Fume hoods must be utilized whenever possible to avoid potential exposure to organic solvents.

All Center for Urban Waters laboratory safety guidelines must be followed, and all laboratory-generated waste must be disposed of according to Center for Urban Waters laboratory waste disposal guidelines.

8 Stocks and Calibration Standards Preparation

8.1 Summary of Stocks and Standards

The D5-6PPDQ ID-ISTD stock solution and the 6PPDQ stock solution are used to prepare the 7-level 6PPDQ calibration standards.

To make 6PPDQ calibration standards, the 6PPDQ stock solution is first diluted to prepare a series of sub-stock solutions. Then, aliquots of sub-stock solution and MeOH are added to autosampler vials to reach the final calibrant concentrations, and an aliquot of the D5-6PPDQ ID-ISTD is added to each calibrant. The individual calibration standards are designated CAL 1 – 7, where CAL 1 is the lowest concentration.

8.2 Requirements

8.2.1 Clean analytical technique

Clean analytical technique is absolutely required. All vessels used in preparation of stocks and standards should be thoroughly cleaned with ethyl acetate and methanol and allowed to dry prior to use. Work should be performed on a clean surface (MeOH-rinsed aluminum foil) with clean, gloved hands. Gloves should be changed as needed to avoid any risk of cross contamination.

8.2.2 Preparation frequency

Stocks should be prepared every 1-2 years, or as needed. Calibration standards should be prepared as needed or every 1-2 months. Stocks and standards should not be used past any of the components' expiration dates.

8.2.3 Storage

After preparation, all stocks and standards should be capped, sealed with parafilm, and stored in the stocks and standards refrigerator in CUW 323.

8.3 Documentation

8.3.1 Autosampler vial label

The following must be documented on the autosampler vial label:

- Compound name
- Compound concentration
- Preparation date
- Initials of preparer

8.3.2 Calibration tracking spreadsheet

The following must be documented in the calibration tracking spreadsheet:

- Mass of all compounds or volume and concentration of all component solutions used to prepare the stock or standard

8.3.3 Laboratory notebook

The following must be documented in the laboratory notebook:

- Unique identification name for stock/standard
- Reagents and standards used, with lot numbers and expiration dates
- Mass of standard added to solution
- Final concentration of solution components
- Preparation date
- Initials of preparer

8.4 Equipment, Reagents, and Supplies

The following equipment/supplies are needed to prepare stocks and calibration standards:

Table 1. Equipment, reagents, and supplies for stocks and standards preparation

Consumables		
Item	**Part Number**	**Vendor**
Optima LC/MS grade Methanol (MeOH)	A456-4	Fisher Scientific
Optima LC/MS grade Ethyl acetate	E196-4	Fisher Scientific
Absolute ethanol (200 proof)	A409-4	Fisher Scientific
D5-6PPD-quinone (100 µg/mL)*	688151	HPC Standards Inc
6PPD-quinone (98.80% purity)**	687855	HPC Standards Inc
Equipment		
20 mL amber glass vials with caps	03-377-41A	Fisher Scientific
Weigh paper	09-89812A	Fisher Scientific
Gas tight syringe set	14-813-142	Hamilton
Sonicator	Branson 5510	Fisher Scientific
Analytical balance	XP205	Fisher Scientific
10 mL Volumetric flask	7064010	Fisher Scientific
1.5 mL autosampler vials with caps	03-391-6/03-391-3	Fisher Scientific

*Liquid solution; stored in main lab freezer in CUW 323
**Solid reference standard; stored in main lab freezer in CUW 323

8.5 Procedure

8.5.1 6PPDQ ID-ISTD Stock Solution Preparation

8.5.1.1 Clean a 10 mL volumetric flask with ethyl acetate and methanol. Then sonicate with ~10 mL of methanol in the sonicator (located in CUW 320) for approximately 10 minutes.

8.5.1.2 Clean a 20 mL amber glass vial with ethyl acetate and methanol.

8.5.1.3 Using a gastight syringe, dispense 10 µL of 100 µg/mL D5-6PPD-quinone ID-ISTD into the 10 mL volumetric flask.

8.5.1.4 Fill the 10 mL volumetric flask with LC-MS grade methanol so that the bottom of the meniscus of the methanol rests on top of the 10 mL mark for the volumetric flask. If the meniscus goes above the mark, start over.

8.5.1.5 Pour the stock solution into the 20 mL amber glass vial. Vials should be capped with new caps with silicone/PTFE septa.

8.5.1.6 Label the vial and document information in the tracking spreadsheet and lab notebook as described in Section 8.3.

8.5.2 6PPDQ Stock Solution Preparation

8.5.2.1 Clean a 50 mL volumetric flask with ethyl acetate and methanol. Then sonicate with ~50 mL of methanol in the sonicator (located in CUW 320) for approximately 10 minutes.

8.5.2.2 Clean a 120 mL amber glass vial with ethyl acetate and methanol.

8.5.2.3 Measure 5 mg of 6PPDQ on the analytical balance. Record exact mass in laboratory notebook.

8.5.2.4 Dispense the solid into the 50 mL volumetric flask. Rinse the weigh paper with Absolute ethanol if needed get entire amount of solid into flask.

8.5.2.5 Using Absolute ethanol, fill the volumetric flask so that the bottom of the meniscus of the ethanol rests on top of the 50 mL mark for the volumetric flask. If the meniscus goes above the mark, you must start over.

8.5.2.6 Ensure that all of the solid material dissolves in the solvent. Facilitate with sonication, if needed. The flask must be sealed with a glass plug and parafilm prior to sonicating.

8.5.2.7 Pour the stock solution into the 120 mL amber glass vial. Vials should be capped with new caps with silicone/PTFE septa.

8.5.2.8 Label the vial and document information in the tracking spreadsheet and lab notebook as described in Section 8.3.

8.5.3 6PPDQ Calibration Set Preparation

8.5.3.1 Label autosampler vials for 6PPDQ Sub-Stock solutions and CALs 1 – 7 as described in Section 8.3.

8.5.3.2 Using a gastight syringe, prepare the 6PPDQ Sub-Stock solutions for each CAL by combining the appropriate volume of 6PPDQ Stock solution or 6PPDQ Sub-Stock solution and MeOH (see **Table 2**) in an autosampler vial.

8.5.3.3 Using a gastight syringe, prepare each CAL standard by combining the required volume of 6PPDQ Sub-Stock solution, 50 µL of the latest D5-6PPDQ ID-ISTD Stock solution and the required volume of MeOH (see **Table 3**) into the appropriate CAL autosampler vial. Cap vials.

8.5.3.4 Label the vials and document information in the tracking spreadsheet and lab notebook as described in Section 8.3.

Table 2. Stock solutions and volumes needed to create 6PPDQ sub-stock solutions.

6PPDQ Sub-Stock Conc. (ng/mL)	Volume of 10 mg/mL 6PPDQ Stock (µL)	Volume of 1000 ng/mL 6PPDQ Sub-Stock (µL)	Volume of 100 ng/mL 6PPDQ Sub-Stock (µL)	Volume of 4 ng/mL Sub-Stock (µL)	Volume of MeOH (µL)
1000	10	--	--	--	990
100	--	100	--	--	900
4	--	--	40	--	960
0.1	--	--	--	25	975

Table 3. Stock solutions and volumes needed to create the 6PPDQ calibrant set.

CAL	Sub-Stock Conc. (ng/mL)	Vol of Sub-Stock (µL)	ID-ISTD Conc. (ng/mL)	Vol of ID-ISTD (µL)	Vol of MeOH (µL)	Final 6PPDQ Conc. (ng/mL)	Final ID-ISTD Conc. (ng/mL)
1	0.1	250	100	50	700	0.025	5
2	4	25	100	50	925	0.1	5
3	4	125	100	50	825	0.5	5
4	100	25	100	50	925	2.5	5
5	1000	10	100	50	940	10	5
6	1000	20	100	50	930	20	5
7	1000	50	100	50	900	50	5

9 Sample Collection and Extraction

9.1 Summary of Sample Extraction

Samples for analysis of 6PPDQ in water are first collected in pre-cleaned glassware. Samples and quality control (QC) samples are then spiked with ID-ISTD to enable quantitation, and extracted using solid phase extraction (SPE).

9.2 Requirements

9.2.1 Clean laboratory and field techniques

Clean laboratory and field techniques are required. Gather and prepare all materials prior to collecting the samples. All sample collection containers and laboratory glassware used in sample extraction should be thoroughly cleaned with ethyl acetate and methanol and allowed to dry prior to use. Gloves should be worn in the field, and laboratory work should be performed on a clean surface (MeOH-rinsed aluminum foil) with clean, gloved hands. Gloves should be changed as needed to avoid any risk of cross contamination.

9.2.2 Solvent waste

Solvent waste produced during this procedure should be disposed of in the appropriately labeled 4 L hazardous waste bottle in the fume hood.

9.2.3 Sample extraction and storage

All sample extraction procedures are performed in the CUW Main Lab (CUW 323).

All samples should be stored in the Environmental Room (CUW 322) at 4°C in the dark until preparation and analysis. Note that some analytes are light sensitive – lights should be off when leaving the environmental room.

Samples must be extracted with 24 h of collection. Extracted samples should be analyzed within 7 days.

9.3 Documentation

9.3.1 Sample collection bottle labels

Sample collection bottle labels must contain:

- Sample ID – unique identifier of date and sample location
- Date of sample collection (time will be noted during sample collection in Field notebook and/or on a chain of custody form)
- Sampler initials

9.3.2 Sample collection records

Sample collection notes should be recorded in the Field notebook for the project and/or on a chain of custody form. The record should include: details of the sample ID, sample location (including map, coordinates, and photos – as appropriate), date and time of sample collection, sample matrix, sample collector, and notes on field conditions (weather and/or other circumstances impacting the collection process).

An example chain of custody form is provided in Section 15.1 (Appendix A).

9.3.3 Autosampler vial labels

Autosampler vial labels must contain:

- Sample ID – unique identifier of date and sample location
- Replicate number
- Date
- Initials of preparer

9.3.4 Sample extraction records

Sample extraction notes should be recorded in the Laboratory notebook for the project and an extraction batch sheet should be completed during sample extraction. The record will include the details of the sample ID and number, sample volume and number of replicates, and associated method/field blanks and QC samples, lot numbers and volumes of reagents and standards used, and the record should be initialed and dated. The batch sheet is printed and written on in indelible ink by the analyst. Mistakes are crossed out with a single line through and initialed/dated by the analyst, with an explanation.

An example batch sheet is provided in Section 15.2 (Appendix B).

9.4 Equipment, Reagents, and Supplies

The following equipment/supplies are needed for sample collection and extraction:

Table 4. Equipment, reagents, and supplies for sample collection and extraction

Consumables		
Item	**Part Number**	**Vendor**
Optima LC/MS grade Methanol (MeOH)	A456-4	Fisher Scientific
Optima LC/MS grade Ethyl acetate	E196-4	Fisher Scientific
Oasis HLB 6cc, 200 mg SPE cartridges	WAT 106202	Waters
Glass beads (filter aid)	EW-35211-50	Cole Parmer
Ultra-pure DI water	W6-4	Fisher Scientific
Equipment		
Item	**Part Number**	**Vendor**
1 L amber glass bottles	89126-606	VWR
Waters Extraction manifold	WAT200606	Waters
15 mL Centrifuge tubes	99502-15	Fisher Scientific
1/8” PTFE tubing	27845	Restek
SEP-PAK RESERVOIR ADAPTOR	WAT054260	Waters
Biotage Turbovap	NC1794836	Fisher scientific
Collection Flask	FB-300-4000	Fisher scientific

Safety Flask	FB-300-1000	Fisher scientific
Vacuum Tubing	14-175H	Fisher scientific
Gas tight syringe set	14-813-142	Hamilton

9.5 Procedure

9.5.1 Glassware Cleaning and Baking

9.5.1.1 Prepare a large sheet of aluminum foil by rinsing with methanol (MeOH) and allowing to try. Clean, un-capped bottles are set on the clean foil on to dry overnight.

9.5.1.2 Rinse bottles, including lids, and glassware with nano-pure DI H_2O, then MeOH, then ethyl acetate, then MeOH. For each rinse, use a squirt bottle to spray down the entire inner surface of the container until there is approximately 25-50 mL of solvent in the container. Be sure to coat every part of the inner surface with solvent. Cap the container and shake thoroughly and dump the solvent into a waste container. Repeat immediately with each reagent.

9.5.1.3 Set clean, un-capped bottles and glassware on the clean foil on to dry overnight.

9.5.1.4 For 1 L amber glass jars: When the bottles are dry (i.e., no liquid is visible inside the jar), they should be capped, the cap should be taped to the body of the jar, and the jar stored until use. Once capped, jars may be stored in cabinets until use and do not need to be further labeled.

9.5.1.5 For other glassware (e.g., collection flasks, centrifuge tubes): When dry, the glassware should then be baked to remove any trace organic contamination from previous uses. place in the glassware furnace for 4+ hours (Figure 1). Glassware should be loaded into the oven with buffer space between glassware for expansion under heat. Once all glassware is placed in the oven, turn the timer knob to 4-5 hours. The timer is located on the wall to the right behind the oven. Once the oven turns on, press the “MODE” button, and make sure that the temperature is set at 450 °C.

9.5.1.6 Once the glassware is done baking and has cooled to room temperature, rinse aluminum foil with MeOH and wrap the open-ended parts of the glassware, or if your glassware is in a beaker, cover the top of the beaker with foil. Once covered with foil, glassware may be stored in cabinets until use and does not need to be further labeled.

Figure 1. Glassware baking furnace. Photo on right shows control timer mounted on wall.

9.5.2 Field Sample Collection

9.5.2.1 Label sample containers individually, using label tape that is subsequently covered with clear packing tape to ensure it does not come off during collection or storage, as described in Section 9.3.

9.5.2.2 Collect sample by first pre-rinsing sample container and container lid 3 times with the water to be sampled. Discard rinse water downstream from sampling location. Samples should be collected with gloved hands to avoid cross contamination and sealed tightly following collection. Additional volumes of water should be collected as needed to enable processing of QC Samples (see **Section 9.5.3**).

9.5.2.3 Samples should be placed in a cooler, on ice, for transport to the laboratory.

9.5.2.4 Record sample collection notes and complete the chain of custody form as described in Section 9.3.

9.5.3 QC Sample Preparation

9.5.3.1 Methanol Blank

The methanol blank is prepared by pipetting 1 mL of the methanol used during sample preparation into an autosampler vial.

9.5.3.2 CCV Standard

The CCV Standard is the CAL-4 standard from the calibration curve.

9.5.3.3 Method Blank

The Method Blank is prepared by extracting 200 mL of DI water, following the procedure described in Section 9.5.4.

9.5.3.4 Field Blank

To prepare Field Blank(s), DI water should be exposed to the same field conditions, equipment, and storage conditions as field samples. The Field Blank(s) are then prepared by extracting 200 mL of this water, following the procedure described in Section 9.5.4.

9.5.3.5 Laboratory Control Sample

The Laboratory Control Samples are prepared by extracting 200 mL of DI water spiked with a known mass of the compounds of interest, following the procedure described in Section 9.5.4.

9.5.3.6 Matrix Spike

The Matrix Spikes are prepared by extracting 200 mL of a representative field sample spiked with a known mass of the compounds of interest, following the procedure described in Section 9.5.4.

9.5.4 SPE

9.5.4.1 Prepare field samples and QC samples that require extraction (method blanks, field blanks, LCS, and matrix spikes)

1. For each field and QC sample replicate, obtain 1 pre-cleaned 1-L amber glass jar. Label the jars with tape and the sample ID.
2. Gently mix the sample so that any suspended solids are evenly distributed. Using a graduated cylinder, measure 200 mL for each replicate and transfer to each 1 L jar.
3. For LCS and Matrix Spike samples, using a clean gastight syringe, add 25 µL of the 6PPDQ Stock Solution to each sample. In the laboratory notebook, record the time, date, name and preparation date of the stock solutions, volume of stock solution added, the sample ID, and the initials of the preparer.
4. For all samples, using a clean gastight syringe, add 50 µL of the D5-6PPD-quinone ID-ISTD stock solution to each sample. In the laboratory notebook, record the time, date, name and preparation date of the stock solutions, volume of stock solution added, the sample ID, and the initials of the preparer.
5. Gently swirl the sample, and then let sit for at least 20 minutes (samples should all be spiked within ~1 h of each other).

9.5.4.2 Manifold preparation and cleaning

1. Assemble the vacuum extraction manifold, 4 L collection flask, and vacuum safety, and attach to vacuum port in hood (**Figure 2**).
2. Clean a portion of lab foil with methanol and set down in fume hood to dry.
3. Assemble the manifold on top of the foil, collection flask, and vacuum safety, and attach the tubing from the vacuum safety to the vacuum port in the fume hood. See **Figure 2**.
4. Clean the tubing with MeOH. Attach straight connectors to manifold and place opposite ends in a clean 400 mL beaker containing clean MeOH (~20 mL/tubing piece). Open all the ports connected to tubing, close all other ports. Turn on vacuum to draw MeOH through hoses and into manifold.
5. Clean the tubing with DI water. Attach straight connectors to manifold and place opposite ends in a clean 400 mL beaker containing DI water (~20 mL/tubing piece). Open all the

ports connected to tubing, close all other ports. Turn on vacuum to draw DI water through hoses and into manifold.

6. Remove all tubing from the manifold, store in clean 400 mL beaker.
7. Clean the manifold with MeOH: Turn on vacuum and squirt MeOH through each open port on the manifold. Clean the top of the manifold with MeOH, and remove the top of the manifold, spray the underside and the interior of the manifold.
8. Turn off the vacuum, and dump the solvent that has collected in the vacuum flask into the hazardous waste container.

9.5.4.3 Prepare and pre-condition SPE cartridges

1. Label each Oasis HLB SPE Cartridges with sharpie (sample ID, replicate number for duplicate samples, date). Set the cartridges on a clean piece of foil as you label them.
2. Add glass beads to each cartridge before placing it on the manifold. If samples are relatively clean, fill glass beads to the bottom of the lower "S" in OASIS. If the samples are relatively dirty, fill with glass beads to the bottom of the upper "S" in OASIS.
3. Place cartridges on the manifold.
4. Close all the ports that have SPE cartridges in them. Leave one un-used port open. Other unused ports should be closed. Process SPE cartridges in batches if there are more cartridges than the total number of ports, minus one.
5. Pre-condition cartridge with MeOH: Fill cartridges with MeOH (~5 mL). Pull through drop-wise. Do this process two times, so a total of ~10 mL passes through cartridge.
6. Connect the tubing to the cartridges (connector snaps into place).
7. Pre-condition cartridges with DI: Place ~25 mL DI water into centrifuge tubes (1 tube per cartridge). Place the tubing for each cartridge into one tube, pull the water through dropwise using the vacuum.

9.5.4.4 Extract sample

1. Place tubing in the respective sample bottles. Use the bottle cap underneath to prop the bottle at a slight angle (to help with getting the last bit of volume, see **Figure 4**).
2. Tape the tubing to the lip of the bottle to hold it in place (the Teflon tubing is jumpy). If the samples are dirty and solids are settled to the bottom, keep the end of the tubing above the bottom of the jar until the very last ~50 mL.
3. When the collection flask fills up, disconnect the vacuum tubing from the inlet to the collection flask & remove the stopper (leave the vacuum on, so that the flow rate does not have to be reset). Dump into sink, then reconnect tubing/stopper.
4. If the cartridges start to clog, tap the side of the cartridge (hard enough to disturb the glass beads).

9.5.4.5 Post-conditioning and drying

1. Remove the tubing from the cartridge.
2. Rinse each cartridge with 10 mL DI (pipet in 5 mL DI twice), dropwise.
3. Vacuum dry cartridges for 15 min.

9.5.4.6 Elution

1. Label glass centrifuge tubes (Turbovap tubes) with sample ID. Set test tubes in the interior of the manifold under the corresponding cartridge (**Figure 4**).

2. Place 5 mL of methanol in cartridge and elute into the corresponding tubes below the cartridges, at a max flow rate of 10 mL/min. Do this process two times, so a total of 10 mL is in the Turbovap tube. Turn off the vacuum when the solvent has been completely drawn through all of the cartridges.
3. If saving cartridges for future elutions (based on project goals), rinse some lab foil with methanol, let it dry in a fume hood, wrap cartridges with the pre-cleaned foil, then label the foil package with labeling tape, include the date, experiment name, and initials of preparer on the label.
4. If needed, eluents can be stored in the freezer: put black screw caps on each Turbovap tube with parafilm around to seal and place the rack in the freezer. Eluents should be evaporated within 48 hours.

9.5.4.7 Evaporation and sample transfer

1. Turn on Turbovap and set water bath temperature to 35 °C and nitrogen pressure to 5 psi. If pressure can't be reached check to make sure that there is air in the tank from the tank room, and replace if needed. If pressure seems to be very sensitive, go to tank room and turn down pressure.
2. Place test tubes into Turbovap and evaporate until ~0.9 mL of eluent remains. Place 0.9 mL of methanol into the same type of centrifuge tube to compare (hold next to each other and compare meniscus) and know when the sample is at 0.9 mL.
3. Label the autosampler vial as described in Section 9.3.
4. Tare the autosampler vial on the scale, and transfer the eluates using a glass Pasteur pipet.
5. Then add clean methanol until the volume is exactly 0.79 g (1 mL).
6. Crimp an aluminum cap onto the autosampler vial.
7. Store in the freezer (4 °C) until analysis.

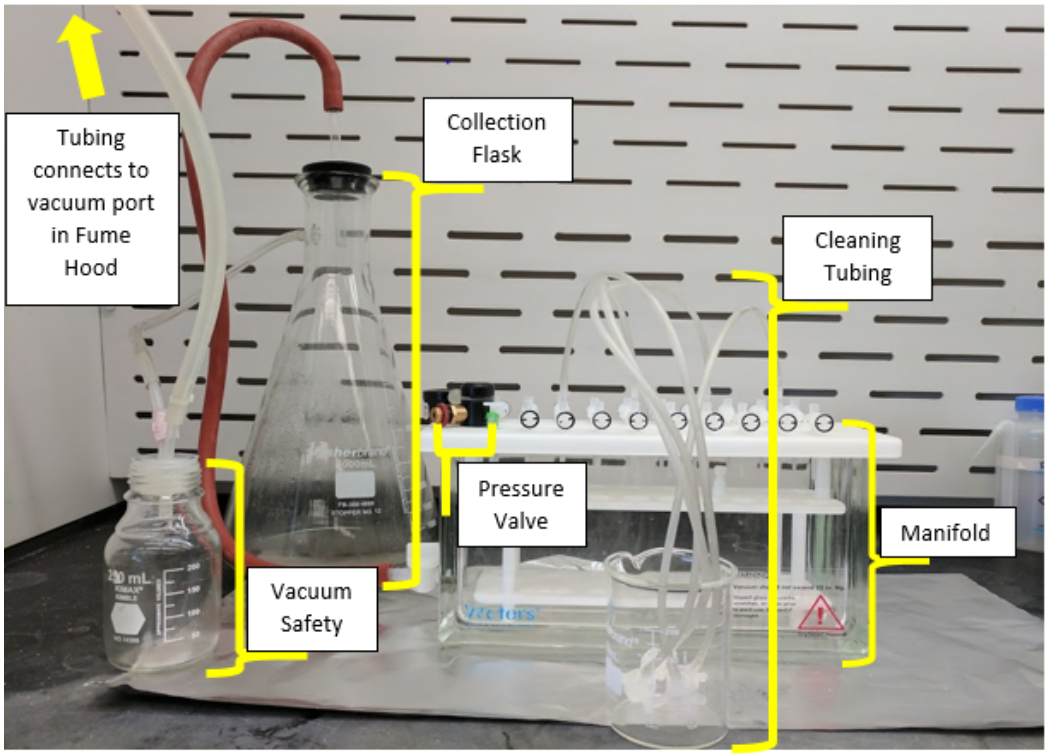


Figure 2. Extraction and elution manifold setup during cleaning.

Figure 3. Extraction manifold setup during sample extraction.

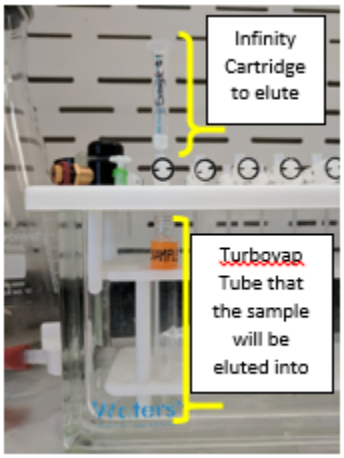


Figure 4. Manifold setup for elution.

10 Sample Analysis

10.1 Equipment, Reagents, and Supplies

The following equipment/supplies are needed for sample analysis:

Table 5. Equipment, reagents, and supplies for sample analysis

Consumables		
Item	**Part Number**	**Vendor**
LC/MS Grade Formic Acid	A117-50	Fisher Scientific
Optima LC/MS Grade Methanol	A456-4	Fisher Scientific
Ultra-Pure DI water	W6-4	Fisher Scientific
Equipment		
Pyrex 1-L bottles	FB-800-1000	Fisher Scientific
1290 Binary pump – Infinity Bin Pump	G4220A	Agilent
1290 Infinity TCC	G1316A	Agilent
1290 Auto Sampler	G4226A	Agilent
QQQ 6460	G6460A	Agilent

10.2 Instrumentation

10.2.1 Instrument Information

The instrument in use at the UWT CUW laboratories is an Agilent 1290 liquid chromatograph (LC) system coupled to an Agilent 6460 Triple Quadrupole (QQQ) mass spectrometer (MS/MS). The specific modules include: a 1290 Infinity Binary pump (G4220A), a 1290 Infinity TCC (G1316A), a 1290 Auto Sampler (G4226A), and a QQQ 6460 (G6460A).

10.2.2 Instrument Maintenance

Instrument maintenance should follow the recommendations of the Agilent 6400 Series LC/MS System Maintenance Guide. Selected key points for preventative maintenance are below:

- **Pump vacuum oil**: check for discoloration and level 1x/month. Change if discolored (dark) or low level. Requires vent of instrument.
- **ESI Capillary:** should be changed/cleaned if significant changes in abundance are observed (e.g., in the CEC standard mix). This requires venting the instrument, should be performed only with Agilent help, and requires having an additional capillary to install during cleaning of the in-use capillary.
- **Source cleaning:** 1x/month
- **Dead pressure of system:** check 1x/month
- **IPA wash of LC side (no column):** 2x/year
- **Connections (tubing):** check for wear 1x/month
- **Solvent frits:** check for discoloration/clogging 1x/month
- **Hydrocarbon trap:** replace 1x / 2 years (or if drop in gas pressure; will have to gauge if 2 years is too short or too long).
- **Guard column:** backflush and/or replace if the pressure goes >100 bar above normal (~650 bar).

10.3 Data Storage

MassHunter acquisition data is collected on the local instrument hard drive (D:\MassHunter\Data\) and then backed up on Dropbox (the current CUW data storage service). All data is stored in a specific user folder the QQQ Data folder. Data files from a single acquisition run should be stored in a unique folder. The format would then be: (D:\MassHunter\Data\Kolodziej\Project\)

10.4 Preparation for Standard Operation

Data acquisition is performed in MassHunter Acquisition Version B.06.00, Build 6.0.6025.4 SP4.

10.4.1 Instrument Pre-Checks

10.4.1.1 Mobile phase

The mobile phase container bottles (1 or 2 L clear glass bottles; cleaned per procedures described in Section 9.5.1) should be made fresh and filled prior to each run. Prepare either 1 or 2 L of each mobile phase. Formic acid should be measured with a gastight syringe; deionized water and methanol are measured with a graduated cylinder. If there is remaining mobile phase from a previous analytical run, it can be used and/or combined with new mobile phases. For the standard acquisition method, use the following:

- Solvent A1: 0.1% Formic acid in deionized water (DI)
- Solvent B1: 0.1% Formic acid in methanol (Optima grade, MeOH)

Once the bottles are filled, update the bottle fill levels in the acquisition software:

- Click on the bottle symbols in the Binary Pump section of the Acquisition view (or right click in binary pump section and select Bottle Fillings).
- Update the volumes (in L) for each solvent bottle.
- Click ok.

10.4.1.2 HPLC column

Check that the correct column is installed. The standard acquisition method utilizes the Agilent Poroshell HPH-C18, 2.7 µm particle size, 2.1 mm x 100 mm employed with a guard column (Security Guard Cartridge, C18, 4 x 2.0 mm ID).

10.4.1.3 Source

The source should be cleaned prior to each analytical run. With a little bit of Isopropanol on a Kimwipe, gently wipe the source around the outer side.

10.4.2 Instrument Initialization

A check-list for instrument set-up is provided in Section 15.3 (Appendix C).

10.4.2.1 Load acquisition method

Load the acquisition method in the method editor window. CUW standard methods are all saved on the control computer drive at: D:\MassHunter\Methods\Kolodziej_Methods\

The method file name for analysis of 6PPDQ is: D:\MassHunter\Methods\Kolodziej_Methods\Melissa\04052021_6PPD Quinone ext gradiant_ISTD_coltemp_left_err.m

Click apply (shown in blue to the right of the method name) (**Figure 5**) and ensure that the same method is shown at the top center dropdown of the Acquisition view. This loads the proper LC and MS/MS settings.

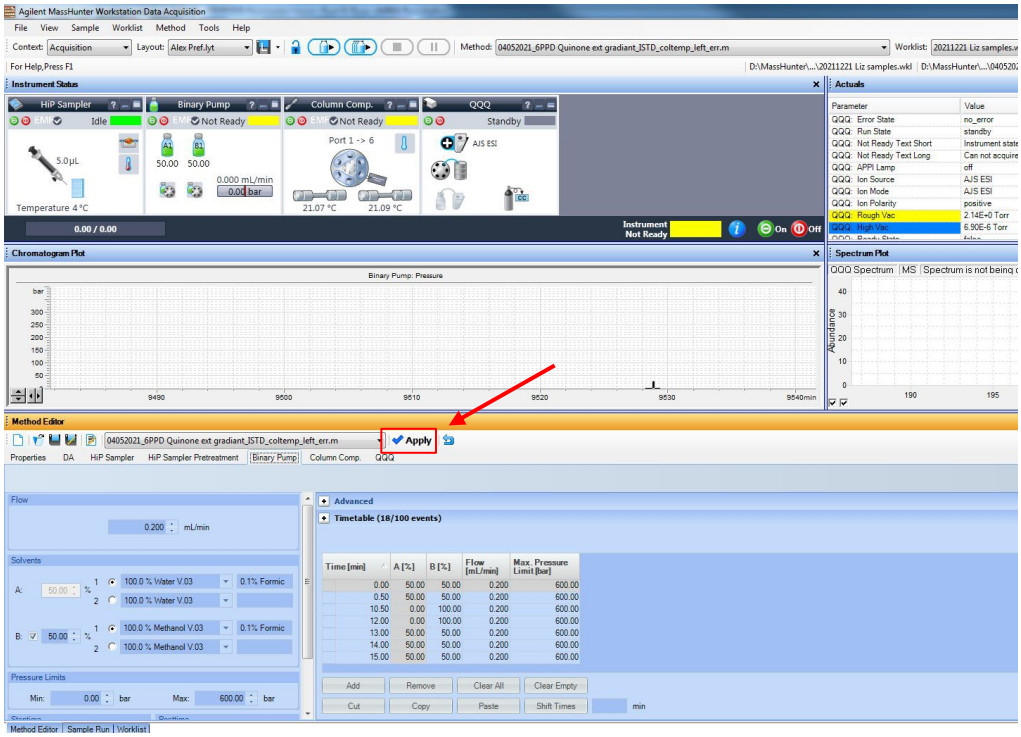


Figure 5. Screenshot of Data Acquisition software, showing the location of the "Apply" buttom to load method settings.

10.4.2.2 Turn on instrument

Purge the binary pump:

- Right click on Binary Pump window, select Control, then change the purge settings to: Duration 3 min, Flow 5 mL/min, bottle B1 50%
- Start Purge: Right click on Binary pump, select Purge on
- The binary pump will be idle at 0.2 mL/min after the purge is complete.

Activate Sampler, Binary Pump, Column Comp, and QQQ by clicking on green button in each systems window (or clicking on larger green button in the bottom right corner of the status display window). See **Figure 6**.

The status of all systems will be listed as Not Ready (yellow) during equilibration. Once ready, all system statuses will change to Idle (green).

Make sure the real-time readout of the MS (right hand side) is visible. If not, turn off the instrument, close the software (put on Standby). Right click on the Rocket Booster icon in the Computer Task bar, and Shutdown engines. Wait 30 seconds after the icon goes red, then re-start the Rocket Booster. Then resume use (re-open software, etc).

The instrument should be allowed to equilibrate prior to tuning and use.

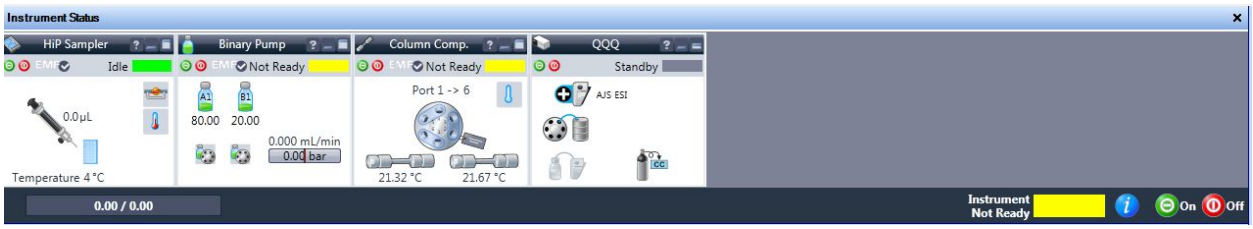


Figure 6. Instrument status bar. Component controls are accessed by right-clicking in each control box. Component status is shown.

10.4.2.3 Check system parameters

Once the Binary Pump is on Idle, check the system pressure and all column and LC fittings for leaks. If pressure doesn't build up, there may be leaks in the system. Expect pressure to settle around ~180 bar. If the pressure is significantly higher (~550 bar), the guard column may require replacement and/or the column or system may require cleaning.

Ensure the ion source interface is on, gas flow is active, and the temperature is ramping up.

Monitor the vacuum levels of the rough vacuum (2.0+0 Torr) and high vacuum (6.75e-06 Torr).

10.4.3 Instrument Tuning

The Agilent 6460 QQQ-MS/MS requires tuning periodically so that one can assess the mass tolerance of the quadrupoles. A standard autotune (see **Figure 7**) is performed using the Agilent Tune Mix that is directly infused into the source from the 6460 QQQ-MS/MS. It is best to perform an autotune individually on each polarity; starting with first positive mode and then another autotune on negative mode.

Prior to every analytical run, a Check Tune is performed typically in both positive and negative modes. The Check tune runs a quick parameter check and tells you the mass and the full width at half maximum (FWHM) are both within tolerance range for both the MS and MS/MS quadrupoles, see **Figure 8** for example of a check tune report. To perform a Checktune or Autotune select "Tune" from the dropdown menu "Context" in the upper left corner. Select "Both" under Polarity and click either "Checktune" or "Autotune." A tune is successful when the result is "Pass" for the all the m/z included in the method. If a Check tune is not successful, a cleaning and an autotune must be performed.

After the check tune, wait for the instrument to re-equilibrate in Acquisition mode for 10 minutes before beginning to run samples.

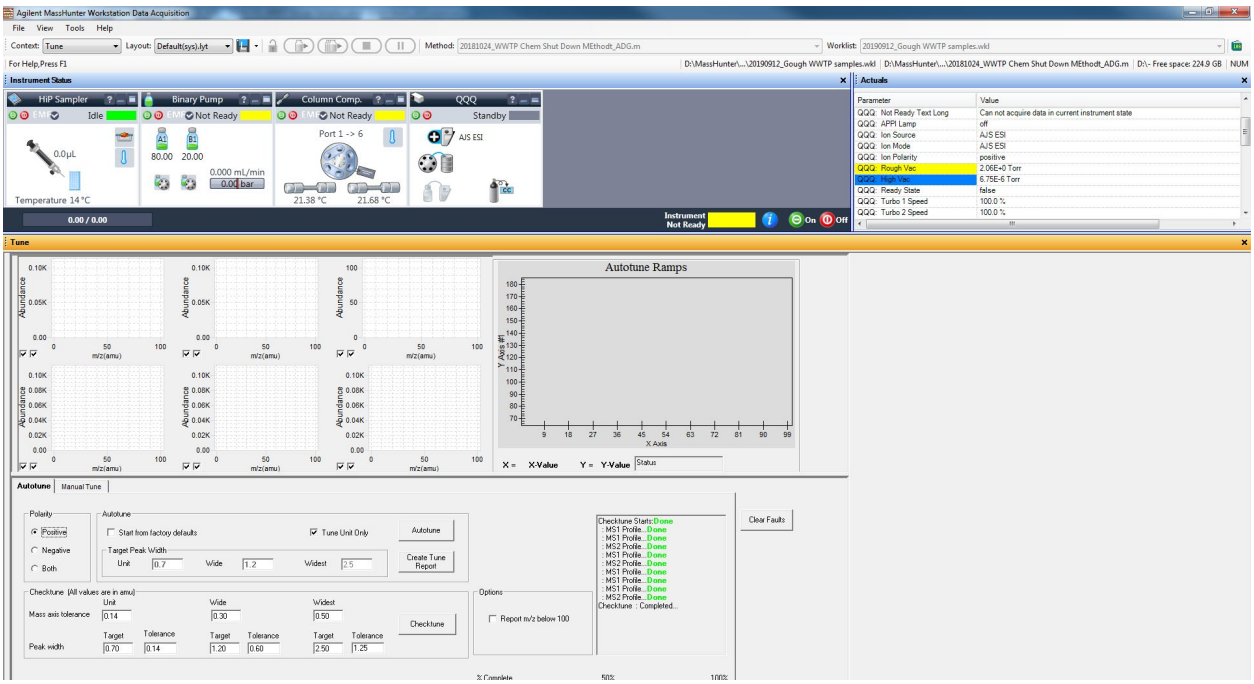


Figure 7. Agilent MassHunter Tune screen

QQQ Check Tune Report

Agilent Technologies

Instrument Name	Instrument 1
MS Model	G6460A
MS Instrument Serial	US93770274
Software_Firmware Version	B.06.00.B6025.4 SP4, FW: A.00.07.32
Tune Date & Time	09:09:19 12:08:14
Data Path	D:\MassHunter\Tune\QQQ\G6460A\atunes.tune.xml
Ion Source	AJS ESI
Ionization Mode	AJS ESI
Tuned Resolution	Unit Only [Wide & Widest Not Tuned]
Vacuum Pressure	1.87E+0[R]; 2.03E-5[H]

Source Parameters

Parameter	Value
Gas Temp	300
Gas Flow	10
Nebulizer	15
Capillary	4000
Nozzle Voltage	1500
Sheath Gas Temp	250
Sheath Gas Flow	7

Positive Results

Analyzer: MS1 Polarity: Positive Width: Unit

m/z Expected	m/z	Tolerance	Delta	Result	FWHM Expected	FWHM	Tolerance	Delta	Result	Abundance
118.09	118.05	0.14	-0.04	Pass	0.70	0.79	0.14	0.09	Pass	262686
322.05	322.06	0.14	0.01	Pass	0.70	0.71	0.14	0.01	Pass	145951
622.03	622.02	0.14	-0.01	Pass	0.70	0.70	0.14	0.00	Pass	343070
922.01	922.00	0.14	-0.01	Pass	0.70	0.70	0.14	0.00	Pass	283697
1521.97	1521.99	0.14	0.02	Pass	0.70	0.68	0.14	-0.02	Pass	102825
2121.93	2121.82	0.14	-0.11	Pass	0.70	0.75	0.14	0.05	Pass	81576

Analyzer: MS2 Polarity: Positive Width: Unit

m/z Expected	m/z	Tolerance	Delta	Result	FWHM Expected	FWHM	Tolerance	Delta	Result	Abundance
118.09	118.10	0.14	0.01	Pass	0.70	0.74	0.14	0.04	Pass	405701
322.05	322.03	0.14	-0.02	Pass	0.70	0.68	0.14	-0.02	Pass	162921
622.03	622.06	0.14	0.03	Pass	0.70	0.67	0.14	-0.03	Pass	260670
922.01	922.02	0.14	0.01	Pass	0.70	0.67	0.14	-0.03	Pass	154404
1521.97	1522.02	0.14	0.05	Pass	0.70	0.68	0.14	-0.02	Pass	71737
2121.93	2122.07	0.14	0.14	Pass	0.70	0.62	0.14	-0.08	Pass	32609

Figure 8. Example check tune report (partial) showing passing tolerances in positive mode.

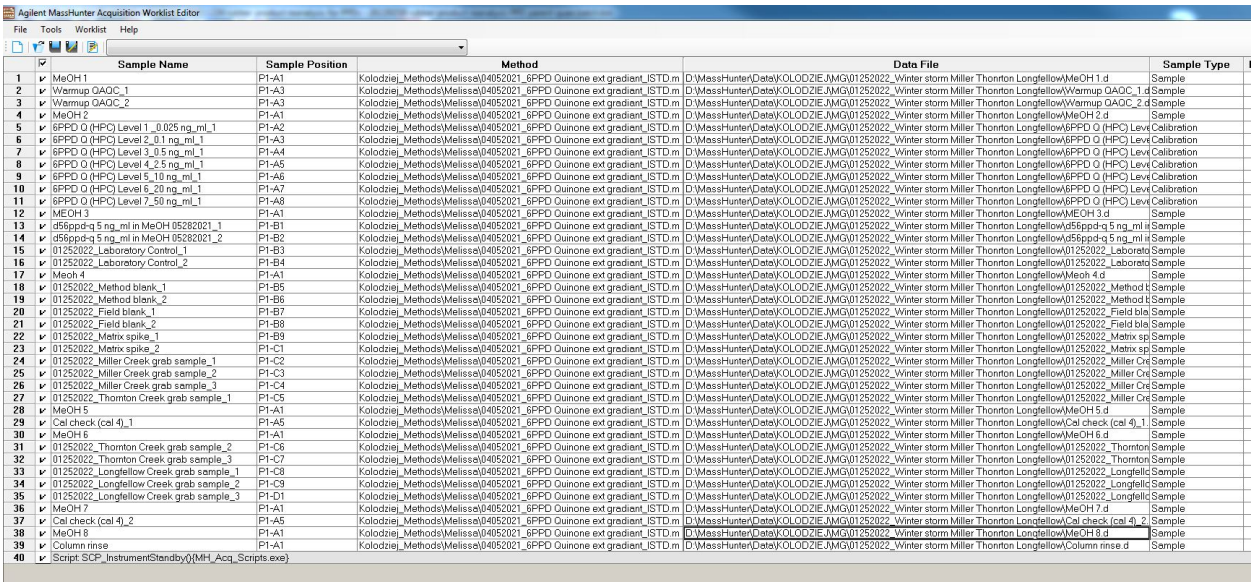
10.4.4 Create Worklist and Start Analysis

The worklist specifies the sample IDs, sample locations (on the sample tray), analytical method, and data file path for each sample in the analytical set. A new worklist may be started, or one can be saved as an existing list under a new name. The worklist editing can be performed either within Acquisition, or in the separate Offline Worklist Editor software. An example worklist is provided in **Figure 9**.

To create a worklist:

1. Worklist Name: Select "Save As Worklist" and save the worklist under a unique name. Filenames include date, project identifier, and initials in format: "YYYYMMDD_ProjectName_Initials"
2. Sample ID: Create an identifier for each sample in the "Sample Name" column of the worklist. Include date and unique identifying details (e.g., YYYYMMDD_SampleName_Replicate#). The same convention is used for QC Samples.
3. Inclusion of QA/QC samples: A methanol blank and CCV (CAL-4) should be run at the beginning and end of each analytical batch. A sequence of methanol blank – CCV – methanol blank should be run every 20-40 samples. The calibration curve should be run at the beginning of the worklist. All other QA/QC samples may be distributed throughout the analytical worklist.
4. A column rinse should be run at the end of the acquisition batch with the method: D:\MassHunter\Methods\Kolodziej_Methods\Fan Hou-2018\Column Rinse_neutral.m
5. Sample loading: Place the autosampler vials in the sample tray. Vials do not need to be modified in any way after completion of sample preparation and may be used directly from the freezer
6. Sample position: Fill in the position of each sample in the sample tray. Example "P1-A1". P1 refers to the position of the entire sample tray in the LC. P1 is the tray position towards the front of the LC. P2 is the tray position to the back. A1 refers to the vial position in the tray. The tray rows are labeled A-F. The tray columns are labeled 1-9.
7. Date file: Create a directory path. Laboratory practice is to save all files under the main data folder, in a subfolder specific to the project. See Section 10.3.
    - Each Acquisition run should be stored in a unique folder. Folder name should be in the format: "YYYYMMDD_BatchName".
    - A unique filename should be specified for each sample – the Sample Name field can be copied into the Data File section.
8. Method: Specify the correct method for each sample.
9. Review the entire worklist for accuracy. Particularly:
    - Each sample has unique sample name
    - The data file path is correct
    - The sample name in the data file path is the same as specified in the sample name.

10. Recheck sample position: Once the acquisition is complete there is no way to re-verify that the sample position in the tray matches the position specified in the worklist. It is imperative that these match at this point. Double check.
11. Select all samples on worklist for analysis. There will be a check in the left edge of the worklist for each sample to be analyzed.
12. Save the worklist after editing.
13. Run analysis by clicking “play” symbol (black triangle).



	Sample Name	Sample Position	Method	Data File	Sample Type
1	MeOH 1	P1-A1	Kolodziej_Methods\Melisse\04052021_6PPD Quinone ext gradient_ISTD.m	D:\MassHunter\Data\KOLODZIEJ\MG\01252022_Winter storm Miller Thonrton Longfellow\MeOH 1.d	Sample
2	Warmup QAQC_1	P1-A3	Kolodziej_Methods\Melisse\04052021_6PPD Quinone ext gradient_ISTD.m	D:\MassHunter\Data\KOLODZIEJ\MG\01252022_Winter storm Miller Thonrton Longfellow\Warmup QAQC_1.d	Sample
3	Warmup QAQC_2	P1-A3	Kolodziej_Methods\Melisse\04052021_6PPD Quinone ext gradient_ISTD.m	D:\MassHunter\Data\KOLODZIEJ\MG\01252022_Winter storm Miller Thonrton Longfellow\Warmup QAQC_2.d	Sample
4	MeOH 2	P1-A1	Kolodziej_Methods\Melisse\04052021_6PPD Quinone ext gradient_ISTD.m	D:\MassHunter\Data\KOLODZIEJ\MG\01252022_Winter storm Miller Thonrton Longfellow\MeOH 2.d	Sample
5	6PPD Q (HPC) Level 1 _0.025 ng_ml_1	P1-A2	Kolodziej_Methods\Melisse\04052021_6PPD Quinone ext gradient_ISTD.m	D:\MassHunter\Data\KOLODZIEJ\MG\01252022_Winter storm Miller Thonrton Longfellow\6PPD Q (HPC) Leve	Calibration
6	6PPD Q (HPC) Level 2_0.1 ng_ml_1	P1-A3	Kolodziej_Methods\Melisse\04052021_6PPD Quinone ext gradient_ISTD.m	D:\MassHunter\Data\KOLODZIEJ\MG\01252022_Winter storm Miller Thonrton Longfellow\6PPD Q (HPC) Leve	Calibration
7	6PPD Q (HPC) Level 3_0.5 ng_ml_1	P1-A4	Kolodziej_Methods\Melisse\04052021_6PPD Quinone ext gradient_ISTD.m	D:\MassHunter\Data\KOLODZIEJ\MG\01252022_Winter storm Miller Thonrton Longfellow\6PPD Q (HPC) Leve	Calibration
8	6PPD Q (HPC) Level 4_2.5 ng_ml_1	P1-A5	Kolodziej_Methods\Melisse\04052021_6PPD Quinone ext gradient_ISTD.m	D:\MassHunter\Data\KOLODZIEJ\MG\01252022_Winter storm Miller Thonrton Longfellow\6PPD Q (HPC) Leve	Calibration
9	6PPD Q (HPC) Level 5_10 ng_ml_1	P1-A6	Kolodziej_Methods\Melisse\04052021_6PPD Quinone ext gradient_ISTD.m	D:\MassHunter\Data\KOLODZIEJ\MG\01252022_Winter storm Miller Thonrton Longfellow\6PPD Q (HPC) Leve	Calibration
10	6PPD Q (HPC) Level 6_20 ng_ml_1	P1-A7	Kolodziej_Methods\Melisse\04052021_6PPD Quinone ext gradient_ISTD.m	D:\MassHunter\Data\KOLODZIEJ\MG\01252022_Winter storm Miller Thonrton Longfellow\6PPD Q (HPC) Leve	Calibration
11	6PPD Q (HPC) Level 7_50 ng_ml_1	P1-A8	Kolodziej_Methods\Melisse\04052021_6PPD Quinone ext gradient_ISTD.m	D:\MassHunter\Data\KOLODZIEJ\MG\01252022_Winter storm Miller Thonrton Longfellow\6PPD Q (HPC) Leve	Calibration
12	MEOH 3	P1-A1	Kolodziej_Methods\Melisse\04052021_6PPD Quinone ext gradient_ISTD.m	D:\MassHunter\Data\KOLODZIEJ\MG\01252022_Winter storm Miller Thonrton Longfellow\MEOH 3.d	Sample
13	d56ppd-q 5 ng_ml in MeOH 05282021_1	P1-B1	Kolodziej_Methods\Melisse\04052021_6PPD Quinone ext gradient_ISTD.m	D:\MassHunter\Data\KOLODZIEJ\MG\01252022_Winter storm Miller Thonrton Longfellow\d56ppd-q 5 ng_ml in	Sample
14	d56ppd-q 5 ng_ml in MeOH 05282021_2	P1-B2	Kolodziej_Methods\Melisse\04052021_6PPD Quinone ext gradient_ISTD.m	D:\MassHunter\Data\KOLODZIEJ\MG\01252022_Winter storm Miller Thonrton Longfellow\d56ppd-q 5 ng_ml in	Sample
15	01252022_Laboratory Control_1	P1-B3	Kolodziej_Methods\Melisse\04052021_6PPD Quinone ext gradient_ISTD.m	D:\MassHunter\Data\KOLODZIEJ\MG\01252022_Winter storm Miller Thonrton Longfellow\01252022_Laborato	Sample
16	01252022_Laboratory Control_2	P1-B4	Kolodziej_Methods\Melisse\04052021_6PPD Quinone ext gradient_ISTD.m	D:\MassHunter\Data\KOLODZIEJ\MG\01252022_Winter storm Miller Thonrton Longfellow\01252022_Laborato	Sample
17	Meoh 4	P1-A1	Kolodziej_Methods\Melisse\04052021_6PPD Quinone ext gradient_ISTD.m	D:\MassHunter\Data\KOLODZIEJ\MG\01252022_Winter storm Miller Thonrton Longfellow\Meoh 4.d	Sample
18	01252022_Method blank_1	P1-B5	Kolodziej_Methods\Melisse\04052021_6PPD Quinone ext gradient_ISTD.m	D:\MassHunter\Data\KOLODZIEJ\MG\01252022_Winter storm Miller Thonrton Longfellow\01252022_Method b	Sample
19	01252022_Method blank_2	P1-B6	Kolodziej_Methods\Melisse\04052021_6PPD Quinone ext gradient_ISTD.m	D:\MassHunter\Data\KOLODZIEJ\MG\01252022_Winter storm Miller Thonrton Longfellow\01252022_Method b	Sample
20	01252022_Field blank_1	P1-B7	Kolodziej_Methods\Melisse\04052021_6PPD Quinone ext gradient_ISTD.m	D:\MassHunter\Data\KOLODZIEJ\MG\01252022_Winter storm Miller Thonrton Longfellow\01252022_Field bla	Sample
21	01252022_Field blank_2	P1-B8	Kolodziej_Methods\Melisse\04052021_6PPD Quinone ext gradient_ISTD.m	D:\MassHunter\Data\KOLODZIEJ\MG\01252022_Winter storm Miller Thonrton Longfellow\01252022_Field bla	Sample
22	01252022_Matrix spike_1	P1-B9	Kolodziej_Methods\Melisse\04052021_6PPD Quinone ext gradient_ISTD.m	D:\MassHunter\Data\KOLODZIEJ\MG\01252022_Winter storm Miller Thonrton Longfellow\01252022_Matrix sp	Sample
23	01252022_Matrix spike_2	P1-C1	Kolodziej_Methods\Melisse\04052021_6PPD Quinone ext gradient_ISTD.m	D:\MassHunter\Data\KOLODZIEJ\MG\01252022_Winter storm Miller Thonrton Longfellow\01252022_Matrix sp	Sample
24	01252022_Miller Creek grab sample_1	P1-C2	Kolodziej_Methods\Melisse\04052021_6PPD Quinone ext gradient_ISTD.m	D:\MassHunter\Data\KOLODZIEJ\MG\01252022_Winter storm Miller Thonrton Longfellow\01252022_Miller Cre	Sample
25	01252022_Miller Creek grab sample_2	P1-C3	Kolodziej_Methods\Melisse\04052021_6PPD Quinone ext gradient_ISTD.m	D:\MassHunter\Data\KOLODZIEJ\MG\01252022_Winter storm Miller Thonrton Longfellow\01252022_Miller Cre	Sample
26	01252022_Miller Creek grab sample_3	P1-C4	Kolodziej_Methods\Melisse\04052021_6PPD Quinone ext gradient_ISTD.m	D:\MassHunter\Data\KOLODZIEJ\MG\01252022_Winter storm Miller Thonrton Longfellow\01252022_Miller Cre	Sample
27	01252022_Thornton Creek grab sample_1	P1-C5	Kolodziej_Methods\Melisse\04052021_6PPD Quinone ext gradient_ISTD.m	D:\MassHunter\Data\KOLODZIEJ\MG\01252022_Winter storm Miller Thonrton Longfellow\01252022_Miller Cre	Sample
28	MeOH 5	P1-A1	Kolodziej_Methods\Melisse\04052021_6PPD Quinone ext gradient_ISTD.m	D:\MassHunter\Data\KOLODZIEJ\MG\01252022_Winter storm Miller Thonrton Longfellow\MeOH 5.d	Sample
29	Cal check (cal 4)_1	P1-A5	Kolodziej_Methods\Melisse\04052021_6PPD Quinone ext gradient_ISTD.m	D:\MassHunter\Data\KOLODZIEJ\MG\01252022_Winter storm Miller Thonrton Longfellow\Cal check (cal 4)_1	Sample
30	MeOH 6	P1-A1	Kolodziej_Methods\Melisse\04052021_6PPD Quinone ext gradient_ISTD.m	D:\MassHunter\Data\KOLODZIEJ\MG\01252022_Winter storm Miller Thonrton Longfellow\MeOH 6.d	Sample
31	01252022_Thornton Creek grab sample_2	P1-C6	Kolodziej_Methods\Melisse\04052021_6PPD Quinone ext gradient_ISTD.m	D:\MassHunter\Data\KOLODZIEJ\MG\01252022_Winter storm Miller Thonrton Longfellow\01252022_Thornton	Sample
32	01252022_Thornton Creek grab sample_3	P1-C7	Kolodziej_Methods\Melisse\04052021_6PPD Quinone ext gradient_ISTD.m	D:\MassHunter\Data\KOLODZIEJ\MG\01252022_Winter storm Miller Thonrton Longfellow\01252022_Thornton	Sample
33	01252022_Longfellow Creek grab sample_1	P1-C8	Kolodziej_Methods\Melisse\04052021_6PPD Quinone ext gradient_ISTD.m	D:\MassHunter\Data\KOLODZIEJ\MG\01252022_Winter storm Miller Thonrton Longfellow\01252022_Longfellc	Sample
34	01252022_Longfellow Creek grab sample_2	P1-C9	Kolodziej_Methods\Melisse\04052021_6PPD Quinone ext gradient_ISTD.m	D:\MassHunter\Data\KOLODZIEJ\MG\01252022_Winter storm Miller Thonrton Longfellow\01252022_Longfellc	Sample
35	01252022_Longfellow Creek grab sample_3	P1-D1	Kolodziej_Methods\Melisse\04052021_6PPD Quinone ext gradient_ISTD.m	D:\MassHunter\Data\KOLODZIEJ\MG\01252022_Winter storm Miller Thonrton Longfellow\01252022_Longfellc	Sample
36	MeOH 7	P1-A1	Kolodziej_Methods\Melisse\04052021_6PPD Quinone ext gradient_ISTD.m	D:\MassHunter\Data\KOLODZIEJ\MG\01252022_Winter storm Miller Thonrton Longfellow\MeOH 7.d	Sample
37	Cal check (cal 4)_2	P1-A5	Kolodziej_Methods\Melisse\04052021_6PPD Quinone ext gradient_ISTD.m	D:\MassHunter\Data\KOLODZIEJ\MG\01252022_Winter storm Miller Thonrton Longfellow\Cal check (cal 4)_2	Sample
38	MeOH 8	P1-A1	Kolodziej_Methods\Melisse\04052021_6PPD Quinone ext gradient_ISTD.m	D:\MassHunter\Data\KOLODZIEJ\MG\01252022_Winter storm Miller Thonrton Longfellow\MeOH 8.d	Sample
39	Column rinse	P1-A1	Kolodziej_Methods\Melisse\04052021_6PPD Quinone ext gradient_ISTD.m	D:\MassHunter\Data\KOLODZIEJ\MG\01252022_Winter storm Miller Thonrton Longfellow\Column rinse.d	Sample
40	Script: SCP_InstrumentStandby(){MH_Acq_Scripts.exe}				

Figure 9. Example worklist

10.5 Acquisition Method

10.5.1 LC Method

This method uses a reverse phase C18 column (Agilent Poroshell HPH-C18, 2.7 µm particle size, 2.1 mm x 100 mm) and C18 guard column (Security Guard Cartridge, 4 x 2.0 mm ID).

The LC method parameters are summarized in **Table** 6 and **Figure 10**, **Figure 11**, Figure 12, and **Figure 13**.

Table 6. LC method parameters

Parameter	Setting
Column temperature	45 °C
Injection volume	5 µL
Flow rate	0.2 mL/min
Eluent A	0.1% formic acid in DI water
Eluent B	0.1% formic acid in methanol

Binary gradient	50% B 0−0.5 min, 50%−100% B 0.5−10.5 min, 100% B 10.5−12 min, 100%−50% B 12−13 min; 50% B 13−15 min

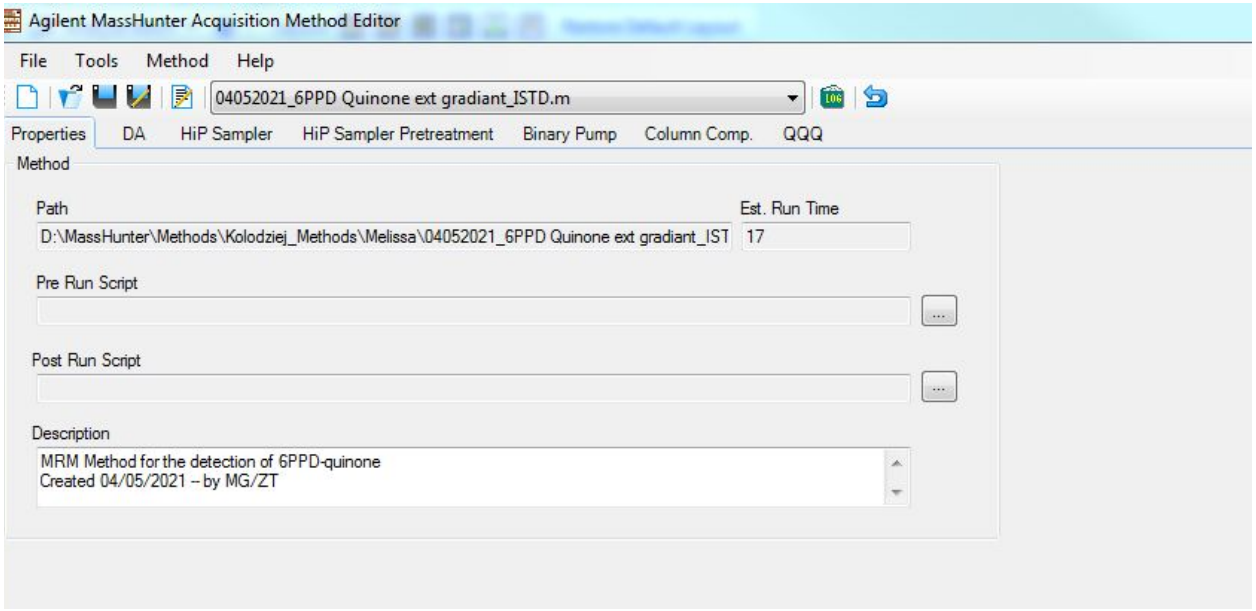


Figure 10. 6PPDQ method properties

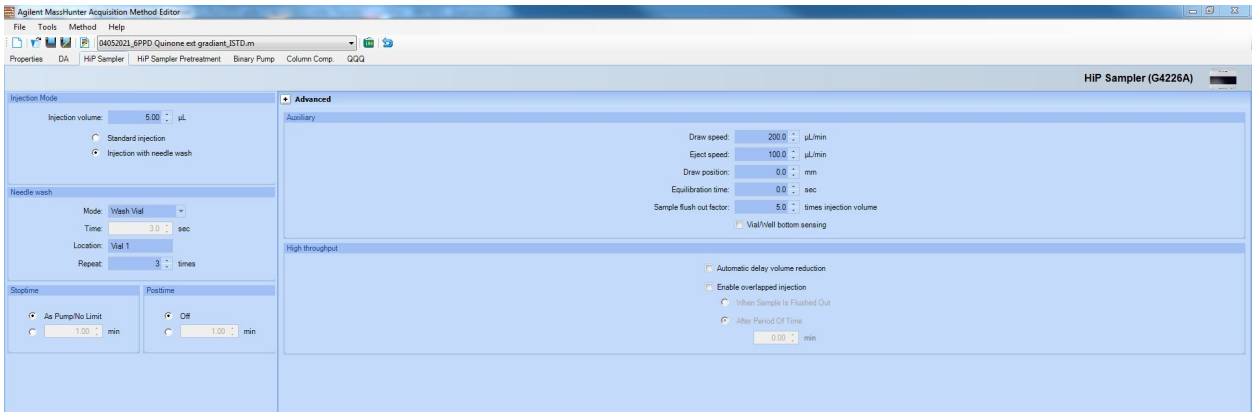


Figure 11. 6PPDQ method autosampler settings

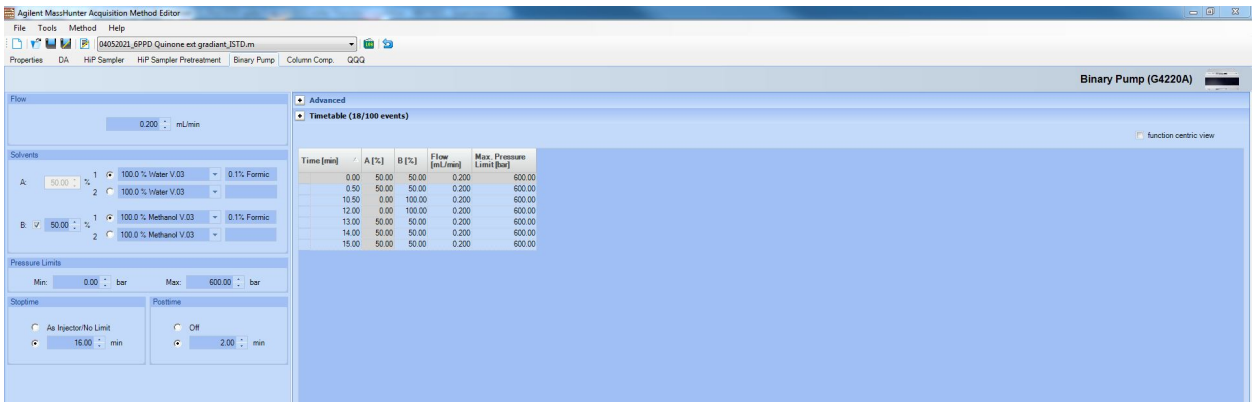


Figure 12. 6PPDQ method LC pump settings

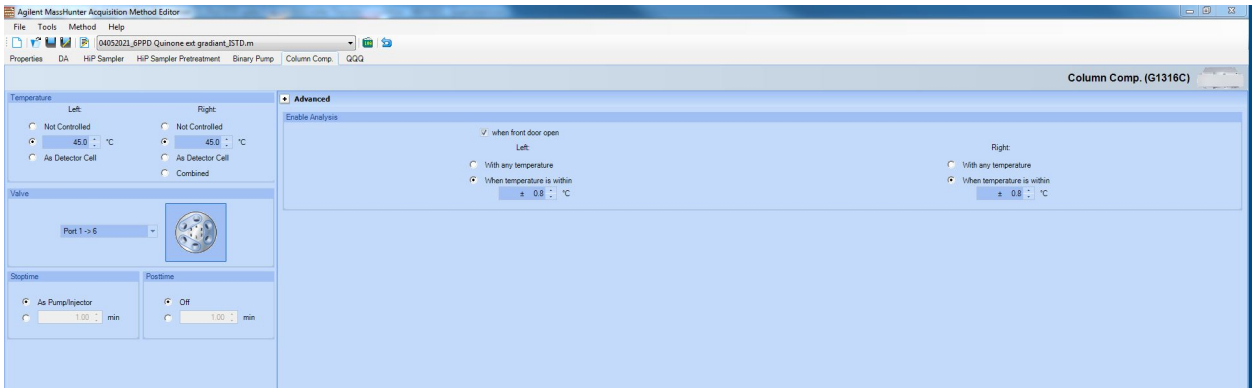


Figure 13. 6PPDQ method LC column settings

10.5.2 MS/MS Method

This method uses electrospray ionization (ESI) and multi reaction monitoring (MRM) for detection. Instrument parameters are summarized in **Table 7**. Pairings of analytes and ID-ISTDs are provided in **Table 8**. Retention times and MS/MS parameters for each analyte and ID-ISTD are summarized in

Table 9 and

Table 10, respectively. MS/MS method settings are shown in **Figure 14**, **Figure 15**, **Figure 16**, and **Figure 17**.

Table 7. MS/MS method parameters

Parameter	Setting
Gas temperature	300 °C
Gas flow	5 L/min
Sheath gas temperature	400 °C
Sheath gas flow	11 L/min
Capillary voltage	3000 V

Nozzle voltage	500 V
Nebulizer	45 psi
Fragmentor voltage	110 V

Table 8. Pairings for target analytes and ID-ISTDs

Target Analyte	ID-ISTD
6PPDQ	D5-6PPDQ

Table 9. MS/MS parameters for target analytes.

Target Analyte	RT (min)	Precursor Ion (*m/z*)	Quantifier Ion (*m/z*)	Quantifier Collision Energy (eV)	Qualifier Ion (*m/z*)	Qualifier Collision Energy (eV)	Fragmentor Voltage (V)	ESI mode
6PPDQ	9.00	299.2	187.1	26	215.1	14	110	Positive

Table 10. MS/MS parameters for isotope dilution internal standards (ID-ISTDs).

Target Analyte	RT (min)	Precursor Ion (*m/z*)	Quantifier Ion (*m/z*)	Quantifier Collision Energy (eV)	Qualifier Ion (*m/z*)	Qualifier Collision Energy (eV)	Fragmentor Voltage (V)	ESI mode
D5-6PPDQ	9.00	299.2	187.1	26	215.1	14	110	Positive

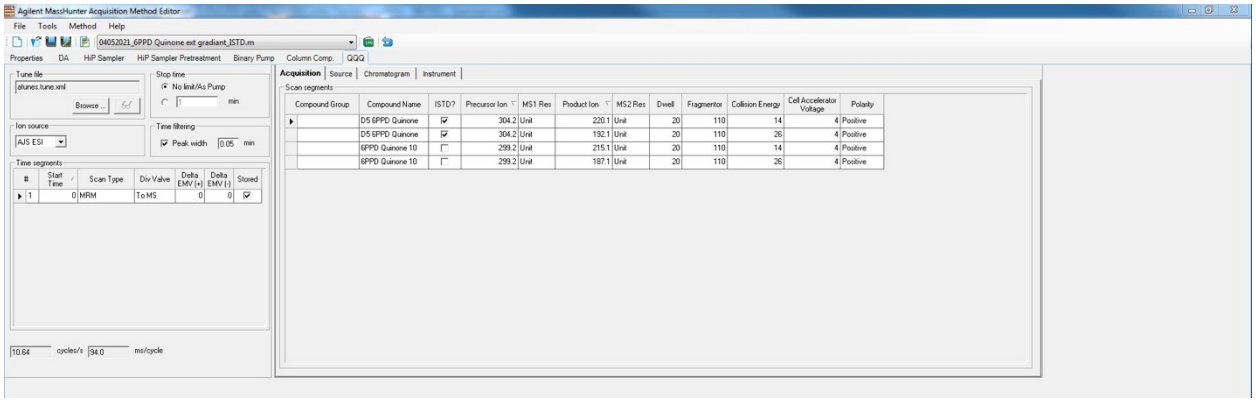


Figure 14. 6PPD-quinone method MS/MS acquisition settings

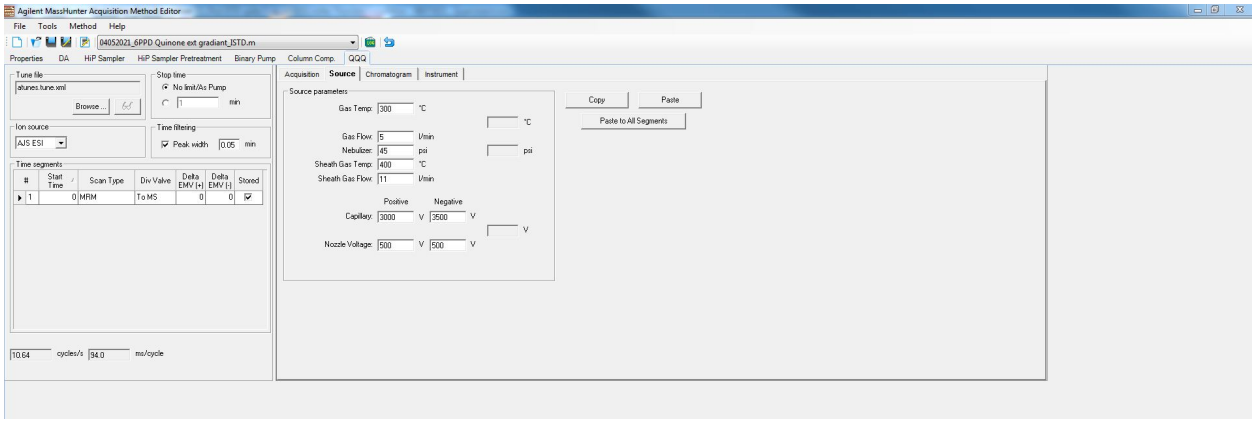


Figure 15. 6PPD-quinone method MS/MS source settings

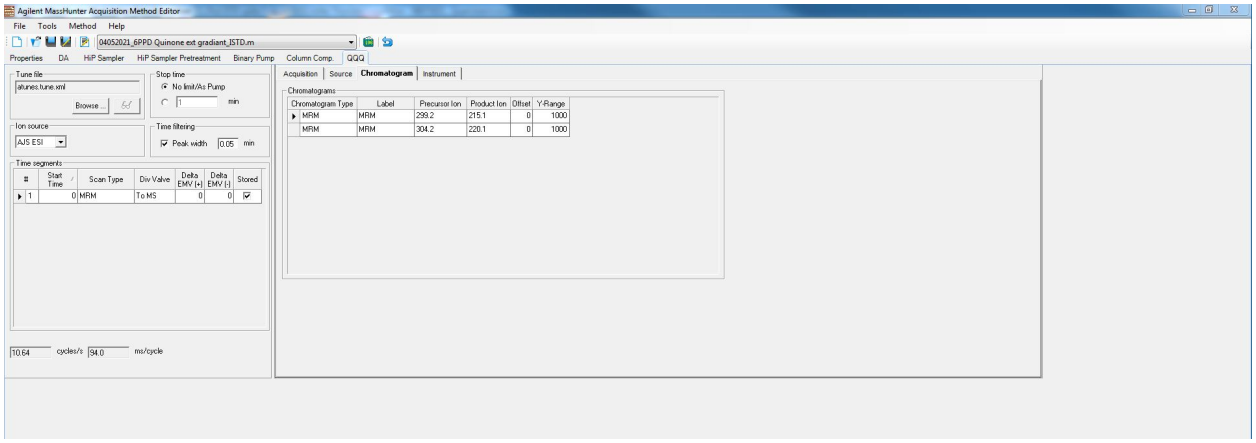


Figure 16. 6PPD-quinone method MRM settings

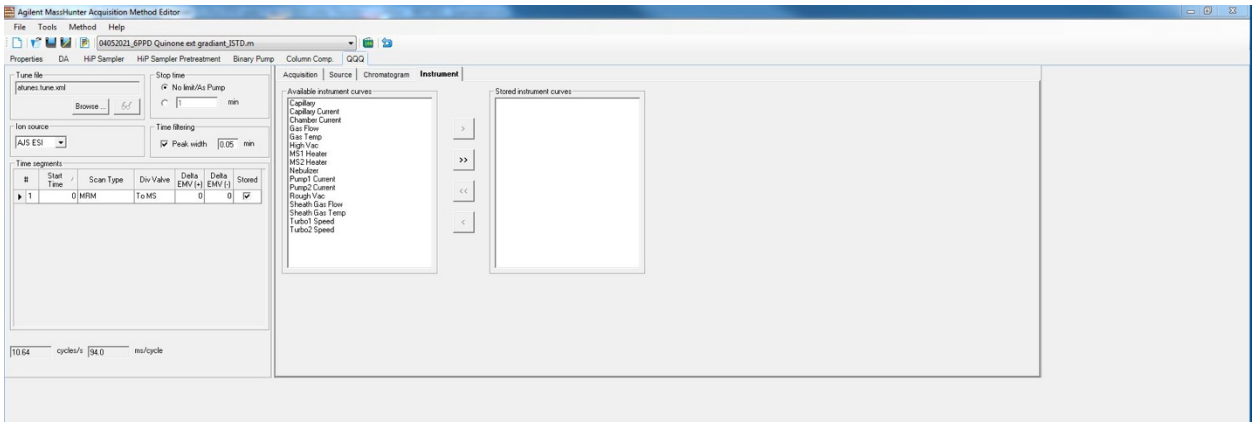


Figure 17. 6PPD-quinone method MS/MS settings

11 Data Analysis

Primary data analysis is performed using Agilent software tools. Data is then exported, with secondary data analysis performed in Microsoft Excel. This section documents the basic processing steps and quality control checks.

11.1 Primary data analysis and export

11.1.1 Software and Analysis Summary

Agilent MassHunter Qualitative Analysis is used for primary data analysis and export. Complete guidance is available on the Agilent MassHunter Workstation Software Qualitative Analysis Familiarization Guide.

For primary data analysis (data quantification), a standard layout has been generated containing all the contaminants of interest. After loading this method in MassHunter Quantitative and selecting the new raw data files from the latest acquisition run, the software extracts the total ion chromatographs (TICs) for MRM transitions and retention time data to identify analytes in samples. The program then automatically integrates the peaks of these transitions and generates peak areas, which is a measure of their quantity. Manual validation of the peak area integrations should be performed to ensure quality control/quality assurance.

The main objectives of the primary data analysis and associated quality control checks are to:

- Create a batch file and calculate concentration in each sample;
- Review the calibration curves for each analyte;
- Review the peaks and peak area for each analyte in each sample;
- Establish a level of confidence in the output by examining QA/QC samples; and
- Export the data for secondary data analysis.

11.1.2 Create Batch File

To create a new batch file:

1. In menu, open: File > New Batch
2. Open the directory containing the sample output files: D:/MassHunter/Data/Kolodziej/
3. Name file with format: YYYYMMDD_ProjectName_Initials
4. In menu, add samples via: File > Add Samples > Select All
5. Open Batch
6. In menu add method: Method > Open > Open and Apply from Existing File, then select method file from folder: D:/Mass Hunter/Quant Methods – select the appropriate method: C:\Users\melisg07\Dropbox (UWT@CUW)\QqQ Data Share\MassHunter Quant Methods\Melissa\05122021_6PPD-quant with ISTD.m
7. Sort the samples based on acquisition time.
8. Label the calibrants as ‘Cal’ instead of ‘Sample’
9. Fill in the appropriate calibrants level indicators (based on their respective cal #) between L1-L7.

10. Click on “Analyze Batch” to apply method (see **Figure 18**). The software will calculate concentrations of analytes of interest in all samples.

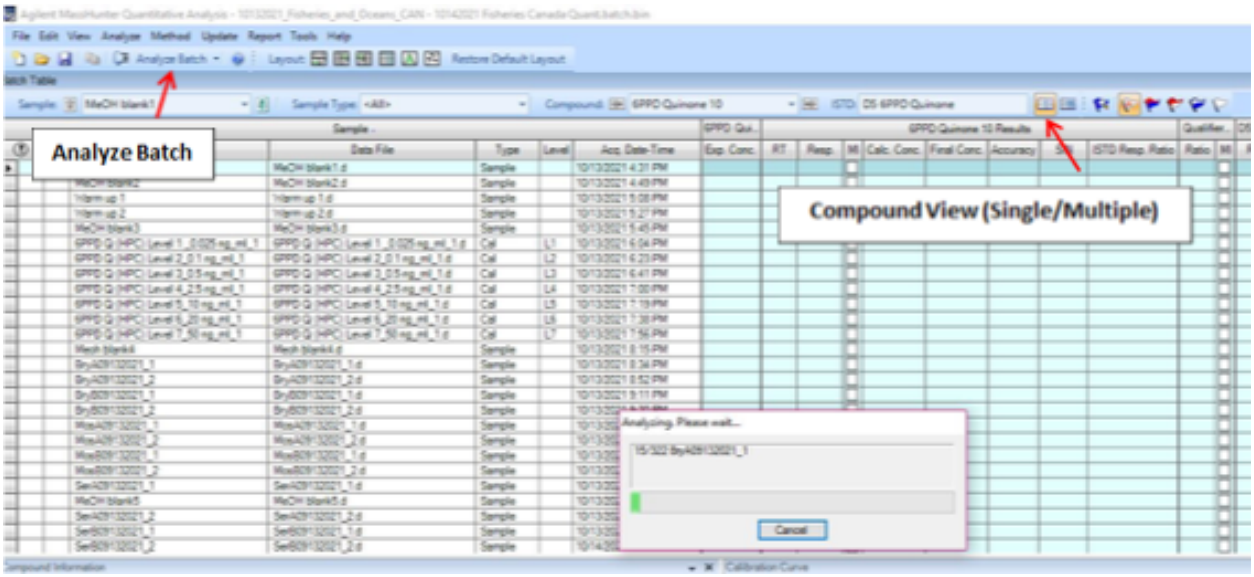


Figure 18. Agilent MassHunter Quantitative Analysis table view highlighting "Analyze Batch" and "Compound View".

11.1.3 Check Calibration Curves

To review the calibration curve for each analyte and verify that a correct and valid peak has been quantified:

1. Change to the view for single compound view and select the analyte of interest. Organize the list based on acquisition time and locate the set of calibrants.
2. Review the calibration curve to determine the goodness of fit of the calibration curve and the suitability of each of the calibrant levels (dilutions) for inclusion in the curve. Calibration models for each compound are provided in **Table 11**. The goodness of fit (R^2) for each regression model should be >0.99.
3. For each calibrant level:
   - Verify the integrated peak for the analyte is correct including peak area and retention time.
   - Verify that the integrated peak for the associated ID-ISTD is correct.
   - The Calculated Accuracy should be between 70-130%.
   - The Response should be significantly greater than in the MeOH blanks.
   - The Response (particularly for the low calibrant levels) should increase with concentration. The same response between calibrant levels suggests that the analyte peak could not be identified.
   - The Response of the calibrant peak should be at least 3x non-calibrant “noise” peaks.
4. Include the widest range of calibrant levels to maintain the $R^2 > 0.99$. The high calibrant defines the maximum quantifiable level. The low calibrant is used in

determining the detection limit. Individual calibrants can be selected/de-selected for inclusion in the regression by clicking on the data point in the calibration curve window. Selected points are solid black circles. De-selected points are hollow circles. No middle calibration curve points can be dropped. Only the end calibration points are allowed to be dropped.

5. Ensure that a sufficient number of calibrants are included in each curve-fit model. EPA SWA-846 Method 8000D recommends the following minimum: 5 points for linear curve-fit model, 6 points for quadratic curve-fit model, and 7 points for other curve-fit models. These minimum recommendations should be followed. Any deviations should be explained in the QA/QC notes.
6. Any adjustments made to the calibrants will immediately be reflected in the goodness-of-fit curve-fit equation. The regression adjustments will not be applied to the calculated concentrations for any of the samples until the batch is re-analyzed. Do so by clicking on **"Analyze Batch."**

Table 11. Calibration model parameters for targeted analytes

Target Analyte	Curve Fit Model	Curve Fit Origin	Curve Fit Weight
6PPDQ	Linear	Ignore	1/x

11.1.4 Check Analyte Peaks in Samples (Qualitative Determination)

To ensure the quantification software integrates the correct peak, ensure the ID-ISTD is correctly quantified, correct any irregular peak integrations, and ensure that integrated peaks are significantly different from noise:

1. Select the analyte of interest. Select samples.
2. Verify that integrated analyte peak has the same retention time as the calibrants and CCVs.
3. Verify that the analyte peak is correctly integrated. The quantification software may incorrectly integrate due to a shifting or irregular baseline (**Figure 19**). A key guideline is that the approach to integrating the peak of interest in the calibrants should be carried over into the sample; consistency is important. Manual integration tools can be used to adjust baselines and beginning and ending time of integration.
4. Verify that the peak integrated by the quantification software is significantly different than background "noise." At a minimum the peak height should be approximately 3x the height of the surrounding peaks.
5. Verify that the peak is quantifiable. A peak response larger than the high calibration is outside the range of quantification. The sample must be diluted and reanalyzed to bring expected concentration within the calibration limits.

6. Verify that the ID-ISTD peak is correctly integrated. The quantification software may occasionally select the incorrect peak. This can be corrected through manual integration tools.
7. Repeat for each targeted analyte.

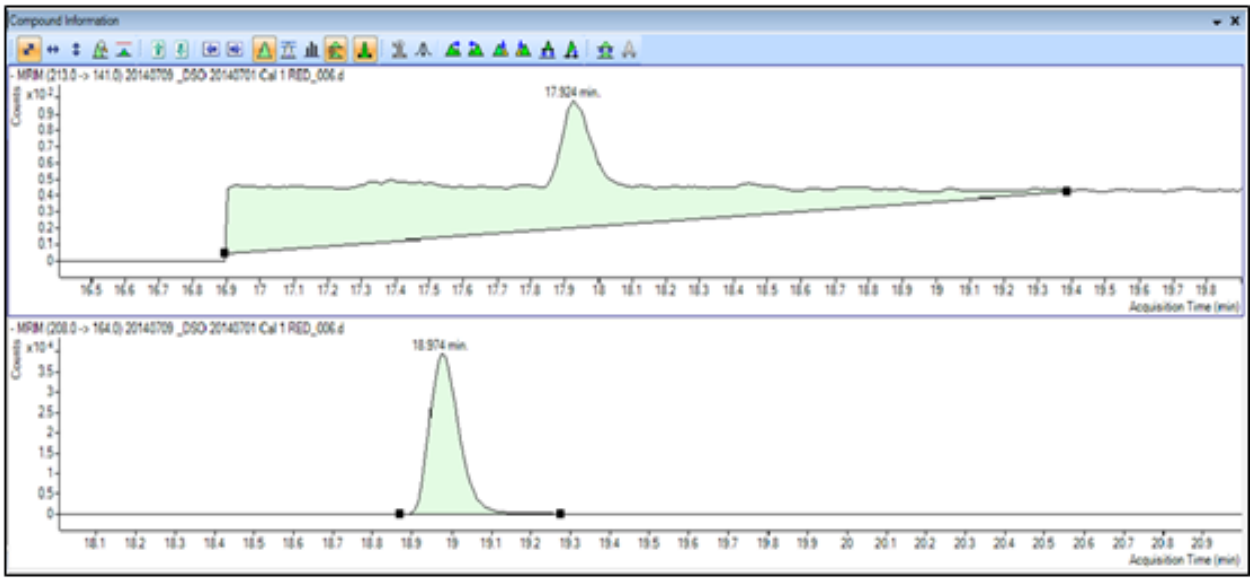


Figure 19. Example of incorrect peak integration by MassHunter Qualitative Analysis software. Peak baseline must be manually corrected.

11.1.5 Check QA/QC Samples

All QA/QC samples (e.g., CCVs, methanol blanks, method blanks, field blanks, LCS, and Matrix Spikes) should be reviewed and quantified in the same manner as field samples.

Method blanks, methanol blanks, and field blanks should be < MDL. If samples are above MDL, the need to re-analyze samples and/or re-evaluate the MDL should be considered.

The calculated accuracy of the CCVs should be between 70-130 %. If the response decreases significantly throughout the run or disappears completely, the cause should be evaluated, corrective action taken as needed, and the samples must be re-analyzed.

11.1.6 Export Data

To export the data for secondary data analysis, set the compound view to "View All Compounds" (**Figure 18**). Export the file by selecting in the menu: File > Export > Export Table > Save as file name.

11.2 Secondary data analysis

11.2.1 Software and Analysis Summary

Microsoft Excel is used for secondary data analysis, and uses the instrumental output exported from MassHunter Quantitative analysis software. QA/QC checks performed during secondary data analysis will be recorded in the 6PPDQ Quantitative Analysis Batch Sheet,

which is included as a sheet in the summarized data file, and is provided in Section 15.4 (Appendix D).

The main objectives of the secondary data analysis are to:

- Create a calculation spreadsheet and organize the data;
- Review the method detection limit for each analyte;
- Calculate final concentrations for each analyte in each sample;
- Establish a level of confidence in the output by examining QA/QC samples and evaluating QA/QC criteria; and
- Format the data for distribution.

11.2.2 Create Spreadsheet and Organize Data

11.2.2.1 Create Calculation Spreadsheet

To create a Calculation Spreadsheet for data analysis: Copy all data in "Sheet 1" to Sheet 2. Rename Sheet 1 "Instrumental Output". Rename Sheet 2 "Data Analysis 1." All subsequent steps will be performed on the Data Analysis sheets; the "Instrumental Output" sheet should remain unchanged.

Additionally, create a Quantitative Analysis Batch Sheet for the analytical batch.

11.2.2.2 Organize Data for Analysis

Re-arrange rows to organize data. Using "Cut Cells" and "Insert Cut Cells" for the entire row of data, arrange Calibrants together, Methanol blanks together, LCS together, samples together, etc. For all samples (other than calibrants, blanks, or controls) order rows in order of acquisition time (oldest to most recent).

Move all duplicate sample results to the row below the appropriate original sample results.

Insert 5 columns to the right of the "Accuracy" column. Label these columns as: "Check upper limit", "Check MDL", "Conc. factor", "Corrected Conc. (ng/L)", and "Uncertainty". In the "Conc. factor" column, enter "200" for every sample.

11.2.3 Evaluate Method Detection Limits

The MDL is stated in **Table 12**. If an analyte is detected above the MDL in the Methanol Blank, Method Blank, or Field Blank, the analyst should note the potential impact on data quality on the Quantitative Analysis Batch Sheet.

Record relevant information regarding method quantitation limits. Insert 3 blank rows below the last Method Blank sample. Title these rows as:

- LOW CAL
- HI CAL
- MDL

For each analyte:

- LOW CAL - enter the value of the concentration of the lowest detected calibrant that was used to create the calibration curve in the MassHunter Quantitative analysis software. This value is recorded in the Quantitative Analysis Batch Sheet.

- HI CAL - enter the value of the concentration of the highest detected calibrant used to create the calibration curve in the MassHunter QQQ Quantification software. This value is recorded in the Quantitative Analysis Batch Sheet.
- MDL – use initial MDL stated in **Table 12**. Record the MDL in the Quantitative Analysis Batch Sheet.

11.2.4 Calculate Final Concentration Results

11.2.4.1 Calculate average concentration and uncertainty for duplicate samples

For any duplicate samples, the data analysis should use the average of the duplicate values. Insert a blank row below each set of duplicate samples. Add the sample name with "_AVG" added to the end in the sample name column. Calculate average values for RT, peak area, ID-ISTD response, etc. from the results of each duplicate sample.

To evaluate uncertainty (required for LCS, optional for other sample types), using the row for the average of the duplicates, calculate the % RPD of the duplicate sample values in the Uncertainty column.

11.2.4.2 Calculate corrected concentration

In the column labeled "Corrected conc. (ng/L)", divide the Sample "Calc. Conc" by the Concentration Factor (200). Then, calculate final concentration in sample by subtracting the average blank concentration from the sample concentration.

11.2.5 Evaluate QA/QC

QA/QC must be included in the evaluation of each batch. There are several criteria that are used to support the usability and reliability of each batch. These are described in full in Section 12. If any of the QA/QC parameters are outside the expected limit, the resulting data should be carefully evaluated by project personnel to ensure usability. Annotations should be applied, as appropriate, to signify the data limitations to the end user.

11.2.5.1 Calbration Limits

The range and accuracy of calibration may be limited by the calibration curve for each analyte. The upper limit of quantification is the HI CAL and should be recorded in the quantification sheet. The lower limit of detection is the MDL and should be recorded in the quantification sheet.

To accurately quantify an analyte in a sample, the analyte mass must be less than the highest calibrant. In the column labeled "Check upper limit," use an IF formula to determine if "Calc. Conc" is greater than the HIGH CAL value. If so, label as "> HI CAL". If possible, dilute and reanalyze samples that are above the HIGH CAL value; quantitative results may then be reported.

To accurately quantify an analyte in a sample, the analyte concentration must be greater than the MDL. In the column labeled "Check MDL", write a formula to test if the analyte concentration is less than the calculated Method MDL. If so, label as "ND".

Further, the coefficient of determination value (R^2) for the regression curves for each analyte must be >0.99 for the range of calibration reported. The quantification of an analyte with a R^2

<0.99 is suspect. As such, the concentration should be reported as “ND” indicating it is Not Detected.

11.2.5.2 CCV Standard

Evaluate the accuracy (i.e., measured vs. actual concentration) of the CCV standard. It should be within 70 – 130% throughout the analytical run.

11.2.5.3 Blanks

Examine the results for the methanol blank, method blank, and field blank. Concentration should be <MDL. If samples are above the MDL, impacts on data quality and usability must be noted.

11.2.5.4 LCS

For the LCS, calculate percent recovery, as detailed in Section 12.8, and evaluate the relative percent difference between duplicate LCS. Criteria for ongoing precision and recovery are provided in

Table 14.

11.2.5.5 Matrix Spike Samples

For the matrix spike samples, calculate percent recovery, as detailed in Section 12.9. Although criteria for matrix spike samples are not set, the analyst should document any observations that may impact data quality on the Quantitative Analysis Batch Sheet.

11.2.6 Format Data for Distribution

Copy and “paste as values” all data from the sheet “Data Analysis 1” into a new sheet in the Quantitative Analysis Batch Sheet for the project. Rename the new sheet “Summary”. Delete all data except for the columns: conc. factor, corrected (average) concentration, check upper limit, and check MDL.

12 Quality Assurance and Quality Control

12.1 Summary of QA/QC Samples

A full set of QA/QC samples must be prepared for each lot number combination used for samples in the batch. For each preparation batch, prepare the following samples, following procedures described in Section 9.5:

- All field samples, including duplicates if necessary for the project as defined by the QAPP
- One Method Blank
- Duplicate Field Blanks, if necessary for the project as defined by the QAPP
- One Laboratory Control Sample and Laboratory Control Sample Duplicate
- One Matrix Spike and Matrix Spike Duplicate, if necessary for the project as defined by the QAPP
- One CCV Standard (CAL-4)
- At least one Methanol Blank

Each analytical batch also requires a calibration curve that is matched to the lot numbers of native and ID-ISTDs used in the preparation batch (see Section 8.5.3 for calibration curve preparation).

12.2 Initial Demonstration of Capability (IDC) Study

The laboratory must make an initial demonstration of the ability to generate acceptable precision and recovery with this method. To do so, analyze four LCS according to the procedures described in this SOP.

Using the results of the set of four analyses, compute the average percent recovery of the concentration of each compound in each extract and the relative standard deviation (RSD) of the concentration for each compound. For each compound, compare RSD and the average percent recovery with the corresponding limits for initial precision and recovery in

Table 14. If RSD and and the average percent recovery for all compounds meet the acceptance criteria, system performance is acceptable, and analysis of blanks and samples may begin. If, however, any individual RSD exceeds the precision limit or any individual average percent recovery falls outside the range for recovery, system performance is unacceptable for that compound. Correct the problem and repeat the test.

12.3 Blind to the Analyst (BTTA) Study

The laboratory must perform two BTTA studies to demonstrate initial method performance. Subsequently, one BTTA study must be submitted per year for all parameters that meet the acceptable requirements for QA/QC. The BTTA study must include all QA/QC elements that would be included with a batch of field samples. The BTTA study successfully demonstrates method performance if the blind spike meets the recovery criteria defined for LCS in **Table 13**. The blind sample included in the BTTA should be spiked by an individual other than the analyst. BTTA samples go through all sample preparation and analysis steps as for field samples. The final data should be reviewed by the individual that prepared the BTTA samples to evaluate whether the data meet QA/QC acceptance criteria.

12.4 Method Detection Limit (MDL) and Method Quantitation Limit (MQL)

The Method Detection Limit (MDL) is the lowest concentration at which an analyte can be detected under routine operating conditions (see 40 CFR 136, Appendix B). The initial MDL is provided in **Table 12**.

The Method Quantitation Limit (MQL) (also referred to as the Lower Limit of Quantitation, LLOQ) is the greater of the lowest calibration point or the average concentration in the Method Blanks plus 10 times the standard deviation of the Method Blanks.

Table 12. Initial MDL in 200 mL water samples

Analyte	Initial MDL (ng/L)	Initial MQL (ng/L)
6PPDQ	0.25	0.8

12.5 Continuing Calibration Verification Standard

A midpoint calibration standard (CAL-4) is used as the CCV standard. The CCV is analyzed at the beginning, end, and every 20-40 samples through the analytical batch to evaluate analytical variability. The criteria for the CCV are provided in

Table 14.

If available, a QC check standard prepared from a second source distinct from the source used to prepare the calibration curve should be analyzed periodically (bi-annually) to assure accuracy of the calibration standards and overall reliability of the analytical process.

12.6 Internal Standards

Quantitation in this SOP uses an isotope dilution method. A labeled analog is spiked into each sample to allow identification and correction of the concentration of the native compounds in the analytical process. To calibrate by isotope dilution, inject the calibration standards, and establish a calibration curve using the relative response of the native and labeled standards, where the x-axis is concentration of the native compound in the calibration standard divided by the concentration of the labeled compound in the calibration standard, and the y-axis is the peak area response of the native compound in the calibration standard divided by the peak area response of the labeled compound in the calibration standard. This is performed in the MassHunter Quantitative Analysis software, as detailed in Section 11, and is identical to the procedure described in EPA 1694, Section 10.4.

12.7 Methanol Blanks, Method Blanks, and Field Blanks

Sample batches include a Methanol Blank and a Method Blank to quantify potential contamination, particularly for ubiquitous compounds. The Method Blanks go through the entire sample preparation process, are also used to calculate percent recovery for the LCS.

Field Blanks may not be necessary or available for every study. If collected, at least duplicate Field Blanks should be processed by the same method as used for samples, and they can be used to assess any contamination stemming from the sample collection effort.

12.8 Laboratory Control Samples

A minimum of two Laboratory Control Samples (LCS) are prepared with every batch of samples. LCS are prepared by spiking a known mass of the compounds of interest into DI water. Each LCS will also be spiked with the isotope dilution internal standard (ID-ISTD). The LCS goes through the entire sample preparation process.

For each of these samples, the LCS spike percent recovery should be determined to evaluate ongoing precision and recovery (OPR).

$$Recovery\ (\%) = \frac{C_{spiked\ sample} - C_{un-spiked\ sample}}{C_{known\ spike}} x100$$

where: $C_{spiked\ sample}$ = analyte concentration in LCS sample
$C_{un\text{-}spiked\ sample}$ = analyte concentration in method blank
$C_{known\ spike}$ = known concentration of analyte spike

The criteria for ongoing precision and recovery are provided in

Table 14.

12.9 Matrix Spikes

If necessary for the project as defined by the QAPP, a Matrix Spike and Matrix Spike Duplicate are prepared with every batch of samples. Matrix Spikes are prepared by spiking a known mass of the compounds of interest into a representative water sample. An appropriate sample is an environmental water sample with a similar or identical matrix to the field samples in the batch (e.g., road runoff, creek water). Each Matrix Spike is also spiked with the isotope dilution internal standard (ID-ISTD). The Matrix Spikes go through the entire sample preparation process.

For each Matrix Spike, the matrix spike percent recovery should be determined to evaluate bias due to potential matrix interference.

$$Recovery\ (\%) = \frac{C_{spiked\ sample} - C_{un-spiked\ sample}}{C_{known\ spike}} x100$$

where: $C_{spiked\ sample}$ = analyte concentration in Matrix Spike samples
$C_{un\text{-}spiked\ sample}$ = analyte concentration in the corresponding un-spiked field sample
$C_{known\ spike}$ = known concentration of analyte spike

12.10 Sample Extraction Duplicates

When possible, given limitations of field sample collections, extraction duplicates should be prepared and analyzed for field samples. At minimum, a duplicate LCS (and a duplicate Matrix Spike, if required for the project) must be prepared and analyzed with each preparation batch.

12.11 Control Charts

If desired, add results from ongoing precision and recovery (OPR) evaluations to QC charts to form a graphic representation of continued laboratory performance. QC charts should be saved on the local instrument hard drive (D:\MassHunter\Data\) and then backed up on Dropbox (the current CUW data storage service).

12.12 Corrective Action

If QC samples do not meet defined acceptance criteria, the analyst will evaluate possible causes. Corrective actions related to sample handling and preparation are detailed in the CUW QA Manual. Additionally, if QC samples do not meet criteria during sample analysis, the analyst will investigate the root cause and correct the issue by, for example, cleaning the LC-MS/MS if there is evidence of diminished peak area response or diluting and re-injecting samples if the samples are above the calibration range.

12.13 QC Acceptance Criteria and Reporting

QC acceptance criteria are summarized in **Table 13**.

Samples with concentration above the highest calibration point (CAL-7) are reported as ">HI CAL" unless diluted and re-analyzed. Samples with concentration <MDL are reported as "ND".

12.14 Minimum Frequency for Conducting all QC Elements and QA/QC Criteria

A full set of QA/QC samples must be prepared for each lot number combination used for samples in the batch. The minimum number of QC samples for each preparation and analytical batch (with corresponding acceptance criteria) are summarized in **Table 13** and **Table 14**, and include a single Methanol Blank, a single CCV standard, a single Method Blank, and duplicate LCS. Additionally, a calibration curve is needed for each analytical batch (matched to the lot numbers of native and ID-ISTDs used in the preparation batch).

Table 13. Summary of QA/QC samples and acceptance criteria. Samples that go through the sample extraction process described in this SOP are denoted with an asterisk (*).

Relevant QA/QC Sample	Quantity per Batch	Acceptance Criteria & Reporting Notation
Calibration Curve	1 (Analyzed at beginning of analytical batch)	• $R^2 > 0.99$ • Calculated Accuracy 70-130% • Retention time consistent with CALs across all samples for each analyte
CCV (CAL-4)	1 (Analyzed at beginning of analytical batch, end of analytical batch, and every 20-40 samples)	• Calculated Accuracy of check standard 70-130% throughout analytical batch • % RSD of all check standards < 30%
Methanol Blank	1 (Analyzed at beginning of analytical batch, end of analytical batch, and every 20-40 samples)	• Concentration <MDL
Method Blank*	At least a one per preparation batch	• Concentration <MDL. If samples are above MDL, impacts on data quality must be noted.
Field Blank*	If required by the project QAPP, at least one per preparation batch	• Concentration <MDL. If samples are above MDL, the MDL may need to be recalculated and/or the impact on data quality should be noted in the reported data
LCS and LCSD*	At least a duplicate per preparation batch	• See **Table 14** for ongoing precision and recovery (OPR) criteria
Matrix Spike and Matrix Spike Duplicate*	If required by the project QAPP, at least a duplicate per preparation batch	• Specific criteria are not set for the Matrix Spike samples; instead, they are used to assess the potential for high or low bias due

		to matrix interference. Analyst documents observations on Quantitative Analysis Batch Sheet.
Field Sample Extraction Duplicates*	If required by the project QAPP, a duplicate for each field sample	• No specific criteria; results of field duplicates are averaged

Table 14. QA/QC criteria for Continuing Calibration Verification (CCV) and Laboratory Control Samples (LCS).

Analyte	CCV	LCS			
	Calculated Accuracy	Initial Precision (RSD)	Initial Recovery	Ongoing Precision (RPD)	Ongoing Recovery
6PPDQ	70 – 130%	30%	60 – 130%	40%	60-130%

13 Records and Data Management

13.1 Sample Documentation

Sample information should be recorded in a paginated laboratory notebook (logbook) and/or chain of custody form, as described in the Data management Section in "The Procedural Manual for the Environmental Laboratory Accreditation Program" Ecology Publication No. 10-03-048 and throughout this SOP.

Sample processing documentation and related observations are recorded in the Extraction Batch Sheets. Data analysis QA/QC and related observations are documented in the Quantitative Analysis Batch Sheets. Batch sheets are printed and written on in indelible ink by the analyst. Mistakes are crossed out with a single line through and initialed/dated by the analyst, with an explanation.

Following the completion of sample collection and processing, all documentation should be initialed and dated, then scanned as a .pdf (or equivalent) and stored in the project computer files. The hard copies of each form should be attached to the laboratory notebook. All notes that are recorded should be dated and initialed the day the information was added. Logbooks used to document project information are retained for at least 5 years.

13.2 Data Storage

Raw data should be kept for at least 5 years, and is stored on the CUW instrument computer and backed up to Dropbox, the current data storage program in use at CUW, and/or backed up weekly to an external hard drive by CUW laboratory personnel. All analysis files are saved within a project folder on the CUW instrument computer and backed up to Dropbox.

14 References

(1) Tian, Z.; Gonzalez, M.; Rideout, C. A.; Zhao, H. N.; Hu, X.; Wetzel, J.; Mudrock, E.; James, C. A.; McIntyre, J. K.; Kolodziej, E. P. 6PPD-Quinone: Revised Toxicity Assessment and Quantification with a Commercial Standard. *Environ. Sci. Technol. Lett.* **2022**, *9* (2), 140–146. https://doi.org/10.1021/acs.estlett.1c00910.

15 Appendices

15.1 Appendix A. Chain of Custody Form

Pg of

CHAIN OF CUSTODY RECORD

University of Washington Tacoma at Center for Urban Waters
326 East D Street Tacoma WA 98421
Email: urbanh2o@uw.edu
Tel: (253) 254-7030

Data Delivery:
☐ Fax #: _____
☐ Email: _____

Customer: _____ Project: _____ Project P.O: _____
Address: _____ Report to: _____
_____ Invoice to: _____
_____ Phone #: _____
_____ Fax #: _____

Client Sample - Information - Identification

Sampler's Signature: _____ Date: _____

SAMPLE #	Sample Identification	Sample Matrix	Date Sampled	Time Sampled	Analysis Request	# of Containers	Notes

Relinquished by:	Accepted by:	Date:	Time:

Comments or Special Requirements:

Cooler:
Temp ___° C
Ice: Yes ☐ No ☐
Ice Pack: Yes ☐ No ☐

Matrix Code
DW=Drinking Water
GW=Ground Water
SW=Surface Water
WW=Waste Water
RW=Raw Water
SE=Sediment
SL=Sludge
S=Soil
SD=Solid
W=Wipe
OIL=Oil
B=Bulk
L=Liquid

Turnaround:
☐ 1 Day*
☐ 2 Days*
☐ 3 Days*
☐ Standard
☐ Other

Data Format
☐ Excel
☐ PDF
☐ GIS/Key
☐ EQuIS
☐ Other

Data Package
☐ Tier II Checklist
☐ Full Data Package*
☐ Phoenix Std Report
☐ Other

15.2 Appendix B. Extraction Batch Sheet

Project name: | Prep batch ID: | Sample type: Water samples

Sample ID*	Sample Type	Before SPE		SPE						Elution	Transfer	Notes
				Pre				Post				
		50 µL ID-ISTD	25 µL Native Std	~0.8cm glass beads	10 mL MeOH	25 mL DI H2O	Sample through SPE	10 mL DI H2O Rinse	N-Evap Dry	10 mL MeOH	Volumize w/ MeOH to 0.79 g	All autosampler vials should be filled to 0.79 g (1 ml). Note weight of MeOH added to reach 1 mL. Document all observations here and in Lab notebook
	Method Blank 1											
	Method Blank 2											
	Field Blank 1											
	Field Blank 2											
	Lab Ctrl Sample 1											
	Lab Ctrl Sample 2											
	Matrix Spike 1											
	Matrix Spike 2											

Confirm balance was calibrated within 1 yr: | SPE type: Waters Oasis HLB 6mL | SPE lot #:

*Sample ID is Date_SampleName_ReplicateNumber where SampleName is the Sample Type or unique identifier for the sampling location

ID-ISTD Mix (Name, Lot#, Expiration Date): | Native Stock (Name, Lot#, Expiration Date):

Methanol lot #:

Analyst Performing Sample Extraction: | Sample Extraction Date: | Batch Sheet Revision: 2023.01.17

15.3 Appendix C. LC-MS/MS Set-up Checklist

LC-MS/MS Setup Checklist			
Batch Name			Notes
Initials			
Date and Time			
Startup	Shutdown and Restart ACQ System Launcher		
	Open Most Recent Method		
Solvent Check	A1: 0.1% Formic acid in DI H2O		
	A2: DI H2O		
	B1: 0.1% Formic acid in MeOH		
	B2: MeOH		
Purge and Prime			
Column Changing & Check	Remove and Plug non-relevant LC column, install relevant LC column		
	Check for leaks		
Turn on TCC and MS			
Turn on Sampler Thermostat @ 4 degrees C			
Update Solvent Levels	A1: 0.1% Formic acid in DI H2O		
	A2: DI H2O		
	B1: 0.1% Formic acid in MeOH		
	B2: MeOH		

15.4 Appendix D. Quantitative Analysis Batch Sheet

6PPD-quinone Quantitative Analysis Batch Sheet

6PPD-quinone Calibration Information

Analyte	Low Cal	Hi Cal	MDL	Notes
6PPD-quinone				

6PPD-quinone QA/QC Check List			
QA/QC Check	**Guidelines**	**Analyst Initials**	**Reviewer Initials**
Calibration Curve	$R^2 > 0.99$		
	Calculated Accuracy 70-130%		
Retention Time	RT values should be consistent across all samples for a given analyte		
Check Standard (mid-level CAL)	Calculated Accuracy 70-130%		
	%RSD < 30%		
Methanol Blank	Concentration <MDL		
Method Blank	Concentration <MDL		
Field Blank (if required)	Concentration <MDL		
MDL Assessment	Samples with concentration above HIGH CAL reported as ">MQL" (unless diluted for re-analysis)		
	Samples with concentration <MDL reported as "ND"		
Laboratory Control Sample	% RPD of LCS replicates <40%		
	% Recovery 60-130%		
Matrix Spike (if required)	Analyst evaluated potential matrix bias impacts		