Quality Assurance Project Plan

6PPD-Quinone Removal in Decant Facility

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Prepared for:

Washington State Department of Ecology

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Planning and Public Works

Pierce County

PLANNING & PUBLIC WORKS

Contents

List of Tables

Quality Assurance Project Plan

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6PPD-Quinone Removal in Decant Facility

1. Abstract

Stormwater catch basins (CB's) play a crucial role in stormwater drainage systems by trapping solid waste, including particles worn off tires. Since these particles are widespread in stormwater runoff, they are likely to accumulate in CB's. As a result, the waste collected during catch basin CB cleaning is likely to be contaminated with 6PPD-quinone (6PPDq).

This project is designed to investigate how effectively 6PPDq is removed during the treatment process at a full-scale Decant facility operated by Pierce County. This facility treats the waste collected from CB's, separating solids and liquids, followed by filtration.

2. Introduction

6PPDq was recently discovered in a groundbreaking study as the chemical responsible for the Urban Runoff Mortality Syndrome (URMS), a phenomenon referring to the mass die-off of adult coho salmon returning to spawn in urban streams in the Puget Sound area, after stormwater runoff (Kendra, et al; Scholz, et al; Tian et al.). The devastating effect of URMS can be explained by the very high toxicity of 6PPDq at low levels of concentration to coho salmon. With a reported LC50 of 95ng/L, 6PPDq is the second most toxic chemical to aquatic species ever evaluated by EPA, after the most toxic chemical Parathion.

6PPDq is the transformation product of 6PPD, an additive in virtually all tires and possibly other rubber products to prevent degradation from ozone. By design, 6PPD migrates to the surface of tires which is oxidized to form a layer of 6PPDq. 6PPD and 6PPDq are released from tires to the environment by rain, vehicle washing, and tire wear particles. These may be carried through stormwater conveyance systems to reach receiving streams.

Reducing 6PPDq in stormwater runoff is urgently needed to protect salmonids and other fishery populations that are central to ecosystems, Tribal rights, and economies of the Pacific Northwest (Ecology). Several adsorption and filtration-based media, which have been approved as Best Management Practices (BMPs) to provide stormwater treatment for the removal of conventional stormwater pollutants, are being actively investigated for the removal of 6PPDq, and bioretention filtration is proven to be highly effective (McIntyre et al).

Activated carbon (AC), with a global market valued at $9 billion, has been widely applied in various industrial sectors and consumer products as an adsorbent to remove a diverse range of pollutants from both air and water. AC's high-performance capabilities stem from its highly porous structure and extensive surface areas, which can reach up to 1000 m^2/g. Theoretically, AC should be highly effective in removing 6PPD-quinone (6PPDq) from stormwater as 6PPDq is a hydrophobic organic compound. However, AC is not commonly used as a BMP for stormwater treatment, and the options for the evaluation of 6PPDq removal in the field are very limited.

3. Background

Pierce County Planning and Public Works (PPW), Maintenance and Operations (M&O) Division, is responsible for managing all County roadways. These responsibilities include the cleaning of stormwater catch basins, debris collection, as well as routine street sweeping. These materials are primarily collected and transported by Vactor trucks which are tank trucks with a heavy-duty vacuum designed to pneumatically load solids, liquids, sludge, and slurry through suction lines.

Dumping Pad – Gravity Drainage of Water

Settling Tank – Primary Solid and Liquid Separation

Flocculation Tank – Chemically Enhanced Solid and Liquid Separation

GAC Filtration –Soluble Organic Removal

Drainage Structure Cleaning Program

M&O maintains about 27,238 drainage structures in unincorporated Pierce County. Drainage structures include catch basins, manholes, catch basin mechanical filters, manhole mechanical filters, up-turned pipes, and curb inlets with catch basins and manholes totaling the vast majority. Drainage structures that are not regularly maintained will not collect sediment and pollutants from stormwater runoff as designed and during flood events could discharge pollutants to waterbodies.

M&O has two teams that inspect a percentage of the drainage structures within County right of way and stormwater ponds on an annual basis. The inspection of catch basins is driven by past inspection and maintenance history. M&O maintains catch basins and manholes to have 12 inches or more of clear space from the lowest pipe invert to the top of the sediment or 25% of the sump is free of sediment, whichever is more. Structures that don't meet this criterion are put on the list for cleaning.

The inspection program typically starts around the second week of March and runs until early summer. Inspection data is processed immediately after collection so that work orders can be generated for cleaning crews. The vactor truck teams start cleaning catch basins in early April and continue until early fall. Inspection and cleaning work occur concurrently during the spring months. The National Pollutant Discharge Elimination System Phase 1 permit requires all drainage structure maintenance work to be completed within six months of inspections. Vactor truck teams are equipped with a mobile data collection device (MDCD) which is typically an iPad or iPhone. The MDCD comes preloaded with the structures that need cleaning along with information that shows the locations of salmon-bearing streams and other critical areas.

Once the structure is cleaned, the teams log a quick assessment of the cleaning activities and then record the work done by function code and date.

After the structures have been cleaned, the vactor truck will return to the decant facility to process the waste materials. At the decant facility, the solid and liquid wastes are separated via pools and a sloped pad. Solid materials are removed from the settling pools and stored until dry enough for hauling. Decanted water will drain through the settling pools into settling moats for further treatment and then to a flocculent tank until it is full. The flocculent tank has a volume of about 10,000 gallons and once it has been filled, 20 pounds of flocculent is added and mixed using a large impellor for 45 minutes. The flocculent is constituted primarily of bentonite with some crystalline silica quartz.

After mixing, sediments will settle to the bottom of the tank and then the decanted water is sent through the GAC filter for final pollutant removal. Decanted water is pumped through the GAC filter between 30 and 40 gallons per minute which is adjustable via a variable speed electric pump. This amount seems to be optimal for the removal of oil, metals, and PAHs while still maximizing the life of the GAC media. The empty bed contact time through the system is about 14 minutes with a flow rate of 35 gallons per minute (GPM). See the calculations below:

Empty Bed Contact Time (EBCT) (Minutes) = Carbon Bed Volume (Gallon) / Flow rate in GPM

[The density of the carbon is between 0.45-0.52 grams per cubic centimeter which gives a mean density of 30 pounds (lbs) per cubic foot where one cubic foot is equal to about 7.48 gallons. The tank capacity

is 2,000 lbs of granular activated carbon. 2,000 lbs divided by 30 lbs/cubic foot equals 66.7 cubic feet which is equal to 498.2 gallons (66.7 x 7.48). The flow rate through the filter ranges between 30 to 40 gallons per minute so we will use 35. 498.7/35 = 14.2 minutes]

On an annual basis, the decant facility discharges about 600,000 gallons of decanted water to the sanitary sewer. It also processes about 2,000 tons of solid materials hauled to regional disposal facilities.

The county's stormwater drainage system uses two main methods to manage rainwater runoff. One method is end-of-pipe treatment BMPs, where pipes carry stormwater to filtration systems or settling ponds. These systems remove pollutants before releasing the water to other waterways or offsite locations. The other method is infiltration, where stormwater soaks into the ground and doesn't reach surface waters. A recent partnership between the county and the University of Washington tested end-of-pipe systems using engineered filters. These filters only reduced 6PPDq by an average of 30%.

4. Project Description

4.1. Project Goal

The goals of this project are:

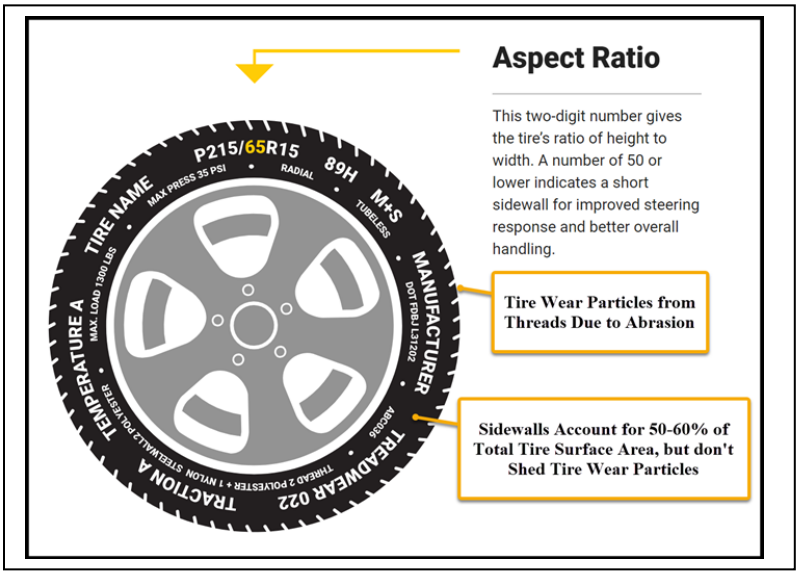
- **Measure the concentration of 6PPDq in stormwater** collected from catch basins (CBs) before it undergoes advanced treatment.
- **Evaluate the effectiveness of the decant facility's treatment processes** (flocculation and GAC filtration) in removing 6PPDq from the stormwater.

While we know 6PPDq comes from tire rubber, the exact ways it enters the environment, how it moves around, and how it ends up in waterways are still unclear.

Looking at tire structure, we can predict two main release mechanisms:

- **Tire wear:** As tires wear down, tiny bits of rubber containing 6PPDq break-off, especially from the tread.
- **Sidewall wash off:** Water washes 6PPDq directly from the tire sidewalls, which don't shed particles.

Understanding these differences is crucial for designing effective ways to reduce 6PPDq in the environment.



Catch basins (CBs) are part of the stormwater system and trap solids. Large tire particles likely settle and get captured in CBs. However, smaller particles and the water-soluble part of 6PPDq probably pass through.

Rain and car washing can release 6PPDq from tire sidewalls. Once on the pavement, it might repeatedly stick to and detach from (adsorb and desorb) various surfaces. Some 6PPDq may travel through stormwater drains, including CBs, and reach waterways. The rest might stay trapped in the CBs, making them a potential storage area (sink) for 6PPDq.

The project initially included mass loading assessment in CB waste. At the time of the proposal being considered, MEL has not been accredited for 6PPDq analysis in sediment. Mass loading assessment is outside the scope of this study, but may be included in future study.

Decant facilities are Ecology's preferred method to dewater street waste. Investigating 6PPDq concentration reductions within the Decant's controlled environment could answer crucial questions and bridge critical data gaps in our quest to reduce 6PPDq in stormwater. Notably, Decant processes decanted water — during the dry season, providing a readily available source of 6PPDq-laden water for study. This avoids the complexities of investigating 6PPDq during unpredictable storm events.

The Decant offers a unique opportunity to address pressing questions about 6PPDq in stormwater. What levels of 6PPDq exist in this concentrated source? Can we effectively remove this harmful compound from decant water? What fraction is removed during the solid and liquid separation steps employed by the Decant? And can GAC treatment reduce soluble 6PPDq below toxic levels, thus safeguarding aquatic life like coho salmon? Deciphering these critical unknowns through controlled studies within the Decant holds promise for advancing our strategies to combat 6PPDq pollution in stormwater.

Furthermore, water quality monitoring data already exist for the Decant facility as it is permitted as a significant industrial user (SIU) by PPW's sewer division, which is subject to the below local limits. County staff will be able to leverage this monitoring requirement to investigate how other pollutants in the Decant water affect the performance of 6PPDq removal.

Table 1. Pierce County Pretreatment Local Limits

Wastewater Parameter	Units of Measurement	Daily Maximum
Arsenic	mg/L	0.23
Cadmium	mg/L	0.11
Chromium (Total)	mg/L	1.00
Copper	mg/L	1.00
Cyanide (Amenable)	mg/L	0.20
Cyanide (Total)	mg/L	0.64
Lead	mg/L	0.40
Mercury	mg/L	0.05
Nickel	mg/L	1.00
Phenol	mg/L	10
Selenium	mg/L	1.45
Silver	mg/L	2.00

Zinc	mg/L	2.00
Fats, Oil & Grease (FOG)	mg/L	100
Total Petroleum Hydrocarbons (TPH)	mg/L	50
pH	S.U.	5.5 -11
Flow	GPD	6,300
Biochemical Oxygen Demand (BOD)	mg/L	
Total Suspended Solids (TSS)	mg/L	

4.2. Project Objectives

1. Conduct 6PPDq sampling and analysis in different stages of stormwater treatment at a Decant facility.

2. Investigate 6PPDq removal by solid/liquid separation and GAC filtration. Investigate interferences by co-contaminants such as TPH, other organics, and suspended solids.

3. Evaluate the hydraulic impact on GAC filtration based on pump rate and empty bed contact time.

- Short-term outcomes

  Determine the effectiveness of solid/liquid separation and GAC media filtration in reducing 6-PPDq at the Decant facility.

- Long-term outcomes

  Decant facility to serve as a platform for 6PPDq-related studies during the off-season.

4.3. Tasks

Task 4.3.1. Procurement of 2000 lbs. GAC

The existing bed of GAC has been in service for over 1 year and removal capacity is likely to have degraded. Projected installation of new GAC in June 2024.

Task 4.3.2. QAPP Development

(Q2 2024) The purpose of this task is to prepare all relevant technical and logistical details and supporting rationale for performing the experiments and for acquiring the data needed for analysis. These study details will be assembled into a Quality Assurance Project Plan (QAPP). The QAPP will be prepared according to Ecology's QAPP template and specifications for projects of this type. The final QAPP will be used by all participants to understand the scope, purpose, approach, and process for performing the study, as well as the role of each person/team in the execution of the project.

Pierce County's subcontractor, the DOE's Manchester Environmental Laboratory (MEL) will describe the methods, protocols, and QA specifications for the analysis and reporting of 6PPDq. Pierce County will be responsible for sample collection, handling, and shipping requirements, and for transporting

treated effluent from the decant facility to MEL.

4.4. Deliverables

D2 Final QAPP Plan
Target Date: June 2024.

Task 4.3.3. Field Work and Laboratory Analysis

(June – October 2024) Task 3 consists of activities to collect stormwater samples biweekly (every 2 weeks) from 3 different locations (see schedule for locations). The decant facility will be operated under normal conditions. Pierce County will perform all field tasks.

This task includes collecting stormwater volumes sufficient for the project and transporting the stormwater samples. Samples from the decant facility will be delivered to DOE Manchester Environmental Laboratory (MEL) according to QAPP specifications for sample timing, delivery, and condition. PPW staff will also transport treated effluent samples from the Decant facility to MEL for 6PPDq analysis, consistent with QAPP specifications.

Pierce County will perform Total Suspended Solids (TSS) /Volatile Suspended Solids (VSS) analysis in all 3 locations using in-house testing facilities.

Pierce County will also leverage existing monitoring requirements to obtain other data to investigate how other co-existing pollutants impact 6PPDq removal.

D3 Lab Analysis Report as Appendix to Final Project Report
Target Date: December 30, 2024

Task 4.3.4. Project Management, Report, and Communication

The Pierce County team will prepare the Project Report for submittal to Ecology with the final billing package by December 30, 2024.

D4.3.4.1 Final Project Report

D4.3.4.2. Public Communication.
Give a presentation of findings to the SWG 6PPD Subgroup.

5. Organization and Project Schedule

5.1. Key Individuals & Responsibilities

Table 2. Roles and Responsibilities

Title	Name	Affiliation	Responsibilities
Ecology Project Manager	**Morgan Baker**	Washington Dept. of Ecology	Coordination for IAA. Provides internal review of QAPP and approves final QAPP. Coordinate with MEL for billing of 6PPDq analysis.
Ecology Quality Assurance Officer	**Chris Dudenhoeffer**	Washington Dept. of Ecology	Reviews/approves draft QAPP and final QAPP.
Pierce County Project Manager	**River Wan**	Pierce County PPW Sewer Division	Contact person for Ecology. Oversees QAPP and development of other deliverables development, and project execution. Responsible for project execution, reporting, and billing.
Pierce County Project Lead	**Jeff Rudolph**	Pierce County PPW M&O Division	Coordination between M&O and Sewer Division for Sampling. Contribute to QAPP. Procurement and installation of GAC.
Pierce County Sampling and Analysis Team	**Amanda Tobin** **Sonia Hernandez** **Earnest Lockett** **Melissa Didier**	Pierce County PPW Sewer Division	Field Sampling/Transportation Local limit sampling and testing TSS/VSS Analysis
MEL Laboratory Project Manager	**Nancy Rosenbower**	Ecology Manchester Environmental Laboratory (MEL)	Coordinate with Pierce County for sample and MEL lab for 6PPDQ analysis. Responsible for preparing chemistry laboratory reports.

5.2. Budget & Schedule

Table 3. Schedule

Calendar Year	2024	2024
Task and Deliverables	Q1-2	Q3-4
1. Procurement of GAC 2000lbs		
D1		
2. Quality Assurance Project Plan		
D2 Final QAPP		
3. Field Work and Laboratory Analysis		
D3 Data Analysis Report – Appendix to Final Report		
4. Project Management and Report		
D4.1 Final Report		
D4.2 Public Communication		

Possible limitations to the proposed schedule are most likely related to fieldwork. Field sampling needs to take place during the decant facility's normal operation. Since the decant facility is operated in batch treatment mode, field sampling staff need to closely coordinate with the decant facility operation staff according to the established sampling schedule to take representative samples.

Table 4. Procurement

Budget		
Tasks	**Description**	**Amount**
1	**Procurement of 2000lbs GAC**	**$5,200**
2	**QAPP Development**	**in-kind**
3	**6PPDq Sampling & Other Field Work**	**in-kind**
	6PPDq Analysis	**$19,500**
	Miscellaneous Analysis (TSS, VSS, Local limits)	**in-kind**
	Supplies (Consumables)	**$2,800**
4	**Project Management (grant management, report preparation)**	**in-kind**
	Total Cost	**$27,500**

6. Quality Objectives

6.1. Data Quality Objectives (DQOs)

This study has no specified regulatory or other standards determining analytical requirements. Quality objectives for this project are defined by those of the laboratories involved and requirements of their internal method protocols, and by the MEL's 6PPD-Q method (Appendix 17.2. Standard Operating Procedure MEL730136, Version 2)

Measurement Quality Objectives (MQOs)

The MQOs for this study are detailed in Table 5.

Table 5. Measurement Quality Objectives

Analytical Method Details - Ecology Manchester Environmental Laboratory											
					Surrogate	Duplicate	Matrix Spike		Blank Spike		
Method	Analyte	MDL	MRL	Units	%R	RPD	%R	RPD	%R	RPD	CAS #
EPA1634	6PPD-quinone	0.480	2.00	ng/L	-	40	50-150	40	70-130	40	2754428-18-5
EPA1634	13C6-6PPD-Quinone			Surrogate	25-200	-	-	-	-	-	
EPA1634	D5-6PPD-quinone			ng/L	-	-	-	-	-	-	

6.2. Targets for precision, bias, and sensitivity

Precision

Precision measures the variability in the results of replicate measurements due to random error. Precision for two replicate samples is measured as the relative percent difference (RPD) between the two results. If there are more than two replicate samples, precision is measured as the relative standard deviation (RSD). Measurement quality objectives for the precision of laboratory duplicate samples and matrix spike duplicate samples are shown in Table 5.

Bias

Bias is the difference between the average measured value and the true value. For this project, bias is calculated as an acceptable % recovery. Acceptance limits for laboratory verification standards, matrix spikes, and surrogate standards are shown in Table 5.

Sensitivity

Sensitivity is a measure of the capability of a method to detect a substance above the background noise of the analytical system. The laboratory reporting limits (RLs) for the project are shown in Table 5.

6.3. Targets for comparability, representativeness, and completeness

Comparability

MEL is the accredited lab for 6PPDq analysis and there will be no splits across multiple labs to verify the comparability between labs.

Representativeness

Representativeness is a measure of whether the sample media reflects reality. We will ensure proper representatives by adhering to the approved SOPs and sampling protocols. Samples will be preserved and stored in a way that ensures holding conditions and lab holding times are met. Samples will be collected to represent the normal operation of the Decant Facility.

Completeness

Given the exploratory nature of this project, the completeness of data collection and analysis will need to be flexible.

6.4. Acceptance criteria for quality of existing data

All data used to support the findings of this project will meet project DQOs.

7. Study Design

7.1. Study Boundaries

The study will be conducted during the typical stormwater CB cleaning season, which runs from May to October each year. This alignment will allow for the collection of data during a period with regular maintenance activities.

7.2. Sample Collection

Table 6. Sampling Locations and Frequency

Decant Facility Study 6PPDq Sampling Plan					
Site	**Locations**	**# of Samples**	**Frequency**	**Duration**	**Total # of Samples**
Decant Facility	Pre-settling Tank	1	Every 2 Weeks	June - October, 2024	10
	Floculantion Tank	1	Every 2 Weeks	June - October, 2024	10
	GAC Filtration	1	Every 2 Weeks	June - October, 2024	10
Total # of Samples					**30**

7.3. Laboratory Analytes to be Measured.

Table 7. Analytes to be Measured at Sampling Locations

Analyte	Sample matrix	Pre-settling Tank	Flocculation Tank	GAC Filtration
6PPD-q	water	X	X	X
TSS	water	X	X	X
VSS	water	X	X	X

TSS = Total Suspended Solids.
VSS – Volatile Suspended Solids

7.4. Assumptions Underlying Design

6PPDq partitions in both solid and liquid phases. Assumptions associated with the study design are that insoluble 6PPD-q is removed in solid and liquid separation processes, but soluble 6PPDq is removed by GAC filtration. The underlying design aims to verify these assumptions by sampling across different treatment processes.

7.5. Possible Challenges and Contingencies

Logistical Problems

Sampling at the Decant facility and sample delivery to MEL requires close coordination between the field sampling team, the Decant M&O staff, and the MEL staff.

Practical Constraints

The practical constraints for this project are having adequate personnel and equipment to support sampling efforts. The 6PPD-q sampling team must balance field sampling with their other responsibilities, including coordination and communication, site accessibility, and upholding safety and quality standards.

8. Field Procedures

8.1. Sampling Procedures

Personnel Qualifications and Responsibilities

- All field staff must be trained in:

    - Standard water quality sampling procedures
    - Collecting representative environmental samples
    - Using appropriate sampling equipment and techniques
- The field staff must be knowledgeable about the project's:
    - Quality Assurance Project Plan (QAPP)
    - Goals and objectives
- All personnel will receive a pre-sampling briefing on the project goals by the Project Manager.

Pre-Sampling Procedures

- Survey sampling locations to identify:
    - Available sampling space
    - Sampling port locations
    - Sampling pole length requirements
    - Safety needs
    - Surrounding area layout
- Label sample containers in advance with the following information:
    - Client
    - Sample ID/Location
    - Date & Time
    - Sampler Initials
    - Parameters to be Tested.
    - Preservatives (if needed)
- Prepare extra labels for potential damage or loss.

Equipment and Supplies

Sampling pole, containers, labels, PPEs

Documentation Procedures

- Document sampling activities using a field log.
- Sampling teams are responsible for completing COC forms for their collected samples.
- MEL will collect COC forms upon sample collection.
- Retain all COC copies for project reference.

Safety

- All sampling events require two personnel to be present.
- Utilize appropriate field safety equipment:
    - Safety vests or highly visible clothing
    - Personal protective equipment (PPE) including:
        - Hardhats (if needed)
        - Goggles (if needed)
        - Earplugs (when needed)
        - Steel-toed boots

- Powder-free gloves

Sample Collection

- Sampling personnel must wear clean, non-talc gloves when handling equipment and containers.
- Grab sampling will occur at the Decant Facility for 4 months at 3 locations.

Sample Storage & Delivery

- Maintain samples between 2℃ and 6℃ until delivery.
- The sampling team will deliver samples to MEL.

Contamination and Interferences

- Minimize contamination from:
  - Sampling equipment, containers, and PPEs
  - Improperly cleaned and stored equipment
  - Atmospheric inputs (dirt, dust)
  - Human contact

8.2. Containers, Preservation Methods, and Holding Times

Table 8. Containers, Preservation, and Holding Time

Lab	Parameter	Matrix	Container or Media	Preservative (°C)	Holding Time (days)
MEL	6PPD-q	water	Certified — 250 mL small mouth amber glass w/ Teflon lid	Cool to 4	28
Pierce County	TSS/VSS	water	1L HDPE container	4 +/- 2	7

HDPE = High-density polyethylene
MEL = Manchester Environmental Laboratory
TSS/VSS = Total suspended solids/Volatile Suspended Solids

8.3. Equipment Decontamination.

To prevent cross-contamination between sampling sites at the decant facility, this protocol outlines a low-transfer approach:

1. **Direct Sampling:** Automatic samplers will be avoided. Instead, pre-cleaned sample bottles will be directly attached to a dedicated sampling pole and submerged into the holding tanks of treated stormwater at each location. This eliminates the need for sample transfer or splitting, minimizing

potential contamination.

2. **Rinsing and Drying:** Between sampling locations, the sampling pole will be thoroughly rinsed with deionized (DI) water and wiped dry with clean paper towels. This ensures residual material from the previous site is removed before collecting the next sample. Chemical cleaning is not necessary with this approach.

3. **Sampling Order:** To minimize carry-over, sampling will follow a specific order based on expected 6PPDq concentration: (1) GAC filtered sample (lowest expected concentration), (2) Flocculation tank, and (3) Pre-settling tank (highest expected concentration).

9. Laboratory Procedures

Table 9. Laboratory Methods

Analyte	Sample Matrix	Analytical Method
6PPD-quinone	Stormwater	Standard Operating Procedure MEL730136, Version 2 (Appendix 17.2)
Total Suspended Solids	Stormwater	SM 2540-D, Pierce County Lab SOP

10. Quality Control Procedures

Table 10. Quality Control Samples, Types, and Frequency.

Parameter	Field Blanks	Field Replicates	Field Method Spikes	Lab Control Sample	Lab Method Blanks	Matrix Spikes
6PPD-q	N/A	N/A	N/A	1/batch	1/batch	2/study
TSS/VSS	N/A	N/A	N/A	1/batch	1/batch	—

Corrective action processes

The laboratory analysts will document whether project data meets method QC criteria. Any departures from normal analytical methods will be documented by the laboratory and described in the laboratory data package and in the project's final report. If any samples do not meet QC criteria, the project manager will determine whether data should be reanalyzed, rejected, or used with appropriate qualifications.

11. Data Management Procedures

Laboratory data package requirements

The laboratory data package will be generated or overseen by MEL. MEL will provide a project data package that will include a narrative discussing any problems encountered in the analyses, corrective actions taken, changes to the referenced method, and an explanation of data qualifiers. Quality control results will be evaluated by MEL.

The following data qualifiers will be used:

"J" — The analyte was positively identified. The associated numerical result is an estimate.

"UJ" — The analyte was not detected at or above the estimated reporting limit.

"NJ" — The analysis indicates the presence of an analyte that has been "tentatively identified," and the associated numerical value represents its approximate concentration.

The qualifiers will be used following the method reporting limits such that:

For non-detect values, MEL is set up to report at the <Reporting Limit Value> with "U".

Only results reported that have a value at least FIVE times the signal-to-noise ratio and meet ion abundance ratios required by the method.

Detected values that are below the quantitation limits (QL) are reported and qualified as estimates ("J").

Results that do not meet ion abundance ratio criteria are reported with "NJ." If an Estimated Maximum Possible Concentration (EMPC) value is calculated and reported, the calculation is explained in the narrative, and an example calculation used for this value is provided.

12. Electronic Transfer Requirements

All laboratory data will be accessed and downloaded from MEL's Laboratory Information Management System (LIMS).

EIM data upload procedures

All completed project data will be entered into Ecology's Environmental Information Management (EIM) database for availability to the public and interested parties. Data entered into EIM follow a formal data review process where data are reviewed by the project manager, the person entering the data, and an independent reviewer.

13. Audits and Reports

13.1. Field, laboratory, and other audits

No defined audit exists for the fieldwork in this project. The Ecology Environmental Laboratory Accreditation Program evaluates a laboratory's quality system, staff, facilities and equipment, test methods, records, and reports. It also establishes that the laboratory can provide accurate, defensible data. All assessments are available from Ecology upon request, including MEL's internal performance

and audits.

13.2. Responsible Personnel

The project manager will be responsible for all reporting.

13.3. Frequency and Distribution of Reports

One final report will synthesize the data and recommend further actions.

13.4. Responsibility for reports

The Project Manager will author the report.

14. Data Verification

14.1. Field Data Verification, Requirements, and Responsibilities

The field team will review field notes. The project manager will provide oversight.

14.2. Laboratory Data Verification

As previously described, MEL will oversee the review and verification of all laboratory data packages analyzed by MEL.

All necessary QC documentation must be provided, including results from matrix spikes, replicates, and blanks.

14.3. Validation Requirements

MEL staff will conduct verification of laboratory data before entering results into the LIMS. Verification will include examining the data for errors, omissions, and compliance with QC acceptance criteria and the method. MEL will include a case narrative that discusses whether (1) MQOs were met, (2) proper analytical methods and protocols were followed, (3) calibrations and controls were within limits, and (4) data were consistent, correct, and complete, without errors or omissions. The case narrative will also define qualifiers and the reason for their use and will be released to the project manager. Laboratory staff may be consulted in order to review QC data that are normally retained by MEL.

The project manager is responsible for the final acceptance of the project data. The complete data package, along with MEL's written report, will be assessed for completeness and reasonableness. Based on these assessments, the data will either be accepted, accepted with qualifications, or rejected and re-analysis considered. The accuracy of data entered into EIM will be verified by someone other than the data engineer per the Environmental Assessment Program's EIM data entry business rules.

Independent validation is not required for this project.

15. Data Quality Assessment

15.1. Process for Determining Project Objectives Were Met

The project manager will determine if the project data are useable by assessing whether the data have met the MQOs. Based on this assessment, the data will either be accepted, accepted with appropriate qualifications, or rejected and re-analysis considered.

15.2. Treatment of Non-detects

Data sums will be qualified with the following:

"J" if that is the only qualifier used.

"NJ" if that is the only qualifier used.

"NJ" if there is a mix of "J" and "NJ" qualifiers.

When all values for individual analytes in the group are reported as non-detects, and the reporting limits are different, the highest value present is assigned as the "total" value. The sum "total" will be qualified with:

"U" if that is the only qualifier used.

"UJ" if that is the only qualifier used.

"UJ" if there is a mix of both "U" and "UJ."

15.3. Data Analysis and Presentation Methods

This study seeks to establish the level of 6PPDq treatment in solid/liquid separation and GAC filtration processes. The percent of solid removal as expressed by % TSS and % VSS removal and percent of 6PPDq removal will be calculated and correlated in each step of the treatment process.

15.4. Sampling Design Evaluation

The sampling design of this project will undergo evaluation between sampling events. The effectiveness of the sample media and our ability to access the necessary sample sites will undergo revision, if necessary, post-reconnaissance.

15.5. Documentation of Assessment

The final report will present this study's findings, interpretations, and recommendations.

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17. Appendices

17.1. Glossaries, Acronyms, and Abbreviations

Glossary of General Terms

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

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.

Dissolved oxygen (DO): A measure of the amount of oxygen dissolved in water.

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

Diurnal: Of, or pertaining to, a day or each day; daily. (1) Occurring during the daytime only, as different from nocturnal or crepuscular, or (2) Daily; related to actions which are completed in the course of a calendar day, and which typically recur every calendar day (e.g., diurnal temperature rises during the day, and falls during the night).

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.

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

Margin of safety: Required component of TMDLs that accounts for uncertainty about the relationship between pollutant loads and quality of the receiving water body.

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.

National Pollutant Discharge Elimination System (NPDES): National program for issuing, modifying, revoking and reissuing, terminating, monitoring, and enforcing permits, and imposing and enforcing pretreatment requirements under the Clean Water Act. The NPDES program regulates discharges from wastewater treatment plants, large factories, and other facilities that use, process, and discharge water back into lakes, streams, rivers, bays, and oceans.

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.

Nutrient: Substance such as carbon, nitrogen, and phosphorus used by organisms to live and grow. Too many nutrients in the water can promote algal blooms and rob the water of oxygen vital to aquatic organisms.

Pathogen: Disease-causing microorganisms such as bacteria, protozoa, viruses.

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.

Phase I stormwater permit: The first phase of stormwater regulation required under the federal Clean Water Act. The permit is issued to medium and large municipal separate storm sewer systems (MS4s) and construction sites of five or more acres.

Phase II stormwater permit: The second phase of stormwater regulation required under the federal Clean Water Act. The permit is issued to smaller municipal separate storm sewer systems (MS4s) and construction sites over one acre.

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.

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 Maximum Daily Load (TMDL): A distribution of a substance in a water body designed to protect it from not meeting (exceeding) water quality standards. A TMDL is equal to the sum of all of the following: (1) individual wasteload allocations for point sources, (2) the load allocations for nonpoint sources, (3) the contribution of natural sources, and (4) a margin of safety to allow for uncertainty in the wasteload determination. A reserve for future growth is also generally provided.

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.

Wasteload allocation: The portion of a receiving water's loading capacity allocated to existing or future point sources of pollution. Wasteload allocations constitute one type of water quality-based effluent limitation.

Watershed: A drainage area or basin in which all land and water areas drain or flow toward a central collector such as a stream, river, or lake at a lower elevation.

303(d) list: Section 303(d) of the federal Clean Water Act, requiring Washington State to periodically prepare a list of all surface waters in the state for which beneficial uses of the water – such as for

drinking, recreation, aquatic habitat, and industrial use – are impaired by pollutants. These are water quality-limited estuaries, lakes, and streams that fall short of state surface water quality standards and are not expected to improve within the next two years.

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
DO	(see Glossary above)
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	(see Glossary above)
GIS	Geographic Information System software
GPS	Global Positioning System
i.e.	In other words
MEL	Manchester Environmental Laboratory
MQO	Measurement quality objective
NAF	New Approximation Flow
NPDES	(See Glossary above)
NSDZ	Near-stream disturbance zones
NTR	National Toxics Rule
PBDE	Polybrominated diphenyl ethers
PBT	Persistent, bioaccumulative, and toxic substance
PCB	Polychlorinated biphenyls
QA	Quality assurance
QC	Quality control
RM	River mile
RPD	Relative percent difference
RSD	Relative standard deviation
SOP	Standard operating procedures
SRM	Standard reference materials
TIR	Thermal infrared radiation
TMDL	(see Glossary above)
TOC	Total organic carbon
TSS	(see Glossary above)
USFS	United States Forest Service
USGS	United States Geological Survey
WAC	Washington Administrative Code
WDFW	Washington Department of Fish and Wildlife
WQA	Water Quality Assessment
WRIA	Water Resource Inventory Area
WSTMP	Washington State Toxics Monitoring Program
WWTP	Wastewater treatment plant

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. For Ecology, it is "Formal recognition by (Ecology)…that an environmental laboratory is capable of producing accurate analytical data." [WAC 173-50-040] (Kammin, 2010)

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* (USGS, 1998).

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: The difference between the sample mean and the true value. Bias usually describes a systematic difference reproducible over time and is characteristic of both the measurement system and the analyte(s) being measured. Bias is a commonly used data quality indicator (DQI) (Kammin, 2010; Ecology, 2004).

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, 1997).

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, 1997).

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: An analyte-specific and sample-specific process that extends the evaluation of data beyond data verification to determine the usability of a specific data set. It involves a detailed examination of the data package, using both professional judgment and objective criteria, to determine whether the MQOs for precision, bias, and sensitivity have been met. It may also include an assessment of completeness, representativeness, comparability, and integrity, as these criteria relate to the usability of the data set. Ecology considers four key criteria to determine if data validation has actually occurred. These are:

- Use of raw or instrument data for evaluation.
- Use of third-party assessors.
- Data set is complex.
- Use of EPA Functional Guidelines or equivalent for review.

Examples of data types commonly validated would be:

- Gas Chromatography (GC).
- Gas Chromatography-Mass Spectrometry (GC-MS).
- Inductively Coupled Plasma (ICP).

The end result of a formal validation process is a determination of usability that assigns qualifiers to indicate usability status for every measurement result. These qualifiers include:

- No qualifier – data are usable for intended purposes.
- J (or a J variant) – data are estimated, may be usable, may be biased high or low.
- REJ – data are rejected, cannot be used for intended purposes.
  (Kammin, 2010; Ecology, 2004).

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, 1997).

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): 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 (USEPA, 1997).

Matrix spike: 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 (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 (EPA, 1997).

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): This definition for detection was first formally advanced in 40CFR 136, October 26, 1984 edition. MDL is defined there as the minimum concentration of an analyte that, in a given matrix and with a specific method, has a 99% probability of being identified, and reported to be greater than zero (Federal Register, October 26, 1984).

Percent 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).

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:

$$[Abs(a-b)/((a + b)/2)] * 100$$

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).

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).

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, 1997).

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, 1997).

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, 1997).

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).

References for QA Glossary

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17.2. Standard Operating Procedure MEL730136, Version 2

Extraction and Analysis of 6PPD-Quinone by EPA 1634

DEPARTMENT OF ECOLOGY
State of Washington

Standard Operating Procedure MEL730136, Version 2

Extraction and Analysis of 6PPD-Quinone by EPA 1634

Approved or Recertified 12/13/2022

Publication Information

The Washington State Department of Ecology develops Standard Operating Procedures (SOPs) to document agency practices related to sampling, field and laboratory analysis, and other aspects of the agency's technical operations.

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Please note that the Washington State Department of Ecology's Manchester Environmental Laboratory (MEL) Standard Operating Procedures (SOPs) are adapted from published methods. They are intended for internal use only and are specific to the equipment, personnel, and samples analyzed at Manchester Laboratory. Our SOPs are not intended for use in other laboratories nor do they supplant official published methods. Distribution of these SOPs does not constitute an endorsement of a particular procedure or method.

Any reference to specific equipment, manufacturer, or supplies is for descriptive purposes only and does not constitute an endorsement of a particular product or service by the author or by the Department of Ecology.

Although the lab follows the SOP in most instances, there may be instances in which the lab uses an alternative methodology or procedure. Deviations to standard procedures must be recorded in pertinent laboratory logbooks and comments sections of the laboratory information management system (LIMS) and ultimately in the case narrative for laboratory reports.

SOP Revision History

Revision Date	Revision History	Summary of changes	Sections	Reviser(s)
12/07/2022	New	Not Applicable	All	Joan Protasio
3/17/2023	1.1	The following changes were made: Section 5.3.5 – added a stipulation that none of the components of a standard solution can be expired Section 6.6.2.1 - added "A minimum frequency of annually." CAS Registry number added to table A01.	5.3.5 6.6.2.1 Table A01	Christina Frans
5/31/2023	1.2	Added explanation for diluted sample concentration calculation and added on column concentration equations.	7.3.2.3	Christina Frans
	2	Modifications to follow EPA Draft Method 1634 (January 2024)	All	Joan Protasio, Jen Pereira

1.0 Purpose and Scope

1.1 This document is Manchester Environmental Laboratory (MEL) Standard Operating Procedure (SOP) for the preparation and analysis of 6PPD-Quinone in water by EPA Draft Method 1634 (January 2024).

1.2 EPA Draft method 1634 is "performance-based," which means that modifications can be made provided that all performance criteria in the method are met.

2.0 Applicability

2.1 This SOP is applicable for 6PPD-Quinone in water. Other analytes and matrices may be added if they meet the minimum QC requirements as outlined in this document.

2.2 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.

3.0 Definitions

3.1 Acronyms

3.1.1	Ecology	Washington State Department of Ecology
3.1.2	EPA	U.S. Environmental Protection Agency
3.1.3	MEL	Manchester Environmental Laboratory
3.1.4	CAS	Chemical Abstracts Service Number
3.1.5	LIMS	Laboratory Information Management System
3.1.6	LLOQ	Lower Level of Quantitation
3.1.7	MRL	Method Reporting Limit
3.1.8	RPD	Relative Percent Difference
3.1.9	RSD	Relative Standard Deviation
3.1.10	RF	Response Factor
3.1.11	SS	Surrogate Standard
3.1.12	EIS	Extracted Internal Standard
3.1.13	NIS	Non-extracted Internal Standard
3.1.14	LC/HPLC	Liquid Chromatography/High Performance Liquid Chromatograph
3.1.15	MS/MS	Mass Spectrometer/Mass Spectrometer (also known as Triple Quadrupole Mass Spectrometer)
3.1.16	SPE	Solid Phase Extraction

3.2 Definitions

3.2.1 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 or Klebsiella.

3.2.2 Calibration: The process of establishing the relationship between the response of a measurement system and the concentration of the parameter being measured.

3.2.3 Continuing Calibration Verification Standard (CCV): A quality control (QC) sample analyzed prior to 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.

3.2.4 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.

3.2.5 Duplicate samples (DUP): Two samples taken from and representative of the same population. The sample and its duplicate are carried through the steps of sampling and analytical procedures in an identical manner. Duplicate samples are used to assess variability of all method activities including sampling and analysis.

3.2.6 Extracted Internal Standard (EIS): An isotopically labeled analog of a target analyte that is structurally identical to a native (unlabeled) analyte. The EIS is added to the sample at the beginning of the sample preparation process and are used to quantify the native target analyte.

3.2.7 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 and is obtained from a second source whenever available.

3.2.8 Initial precision and recovery (IPR): Four aliquots of a reference matrix spiked with the analytes of interest and labeled compounds and analyzed to establish the ability of the laboratory to generate acceptable precision and recovery. An IPR is performed prior to the first time this method is used and any time the method or instrumentation is modified.

3.2.9 Limit of Quantitation (LOQ): The smallest concentration that produces a quantitative result with known and recorded precision and bias. The LOQ is set at or above the concentration of the lowest initial calibration standard (the lowest calibration standard must fall within the linear range).

3.2.10 Lower Limit of Quantitation (LLOQ): The lowest point of quantitation, which, in most cases, is the lowest concentration in the calibration curve.

3.2.11 Matrix Spike (MS): 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 (Ecology, 2004).

3.2.12 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.

3.2.13 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.

3.2.14 Method blank (MB): A blank prepared to represent the sample matrix, prepared and analyzed with a batch of samples. A method blank must contain all reagents used in the preparation of a sample, and the same preparation process is used for the method blank and samples.

3.2.15 Method Detection Limit (MDL): The MDL is defined in 40CFR-136-B as the minimum measured concentration of a substance that can be reported with 99% confidence that the measured concentration is distinguishable from method blank results.

3.2.16 Ongoing precision and recovery standard (OPR): A method blank spiked with known quantities of analytes. The OPR is analyzed exactly like a sample. Its purpose is to assure that the results produced by the laboratory remain within the limits specified in this method for precision and recovery.

3.2.17 Precision: The extent of random variability among replicate measurements of the same property; a data quality indicator.

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

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

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

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

3.2.22 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.

4.0 Personnel Qualifications/Responsibilities

4.1 The analysis in this method is restricted to use by or under the supervision of chemists experienced in the use of liquid chromatography mass spectrometry/mass spectrometry (LC/MS/MS) and the interpretation of chromatograms and mass spectra.

4.2 Training in this procedure with experienced personnel and completion of the training checklist and IDCs are recommended.

4.3 This analysis is typically performed by a Chemist 3 or Chemist 4.

5.0 Equipment, Reagents, and Supplies

5.1 Equipment

5.1.1 Liquid chromatography triple quadrupole mass spectrometer system (LC-QQQ). This system contains an octopole guide to focus the ions toward quadrupole 1 which is MS1, this is for the precursor ions. The second quadrupole is really a hexapole used as a collision cell. A hexapole is used here because it improves focusing like a quadrupole and ion transmission like an octopole. The third quadrupole is MS2; this is for the product ions.

5.1.2 LC - a system with gradient programming, injection control and interface to a mass spectrometer. Agilent model 1260 or 1290 HPLC system capable of performing gradient adjustments at a constant flow rate or equivalent.

5.1.3 Agilent model 6460 or Ultivo Triple Quadrupole Mass Spectrometer (LC-QQQ) with an electrospray Ion Source using jet stream technology (ESIJT) - capable of scanning from 50 to 300 m/z every 0.5 sec or less or equivalent.

5.1.4 Agilent MassHunter data acquisition and processing system - capable of controlling the LC-QQQ and the continuous acquisition of all mass spectra and ions obtained throughout the duration of the chromatographic program.

5.1.5 Analytical column – Reverse phase LC column 100 mm x 2.1 mm ID with 2.6 um Biphenyl 100 Å packing capable of baseline separation of the target compounds (Phenomenex 00D-4622-AN or equivalent).

5.1.6 Guard column (optional) – ZORBAX Extend C-18 4.6 mm, 1.8 um, UHPLC guard column (Agilent 820750-906)

5.2 Reagents

5.2.1 Milli-Q water – 18 megohms or better, free of organic contaminants.

5.2.2 Methanol - HPLC grade or equivalent.

5.2.3 Acetonitrile- HPLC grade or equivalent.

5.2.4 Hexane- Pesticide grade or equivalent.

5.2.5 Formic Acid – ACS grade or equivalent.

5.2.6 Organic reagent (Acetonitrile with 0.1% Formic Acid) – Add 1mL Formic Acid to a final volume of 1L of Acetonitrile. Reagent can be purchased premade.

5.2.7 Aqueous reagent (Water with 0.1% Formic Acid) – Add 1mL Formic Acid to a final volume of 1L of Milli-Q water. Reagent can be purchased premade.

5.3 Standards

5.3.1 Isotopically Labeled Standards:

5.3.1.1 D5-6PPD-Quinone: HPC Standards 688151 or Cambridge Isotopes DLM-11618. Store according to vendor specifications. This is used as the Non-extracted Internal Standard (NIS).

5.3.1.2 13C6-6PPD-Quinone: Cambridge Isotopes CLM-12293 or equivalent. Store according to vendor specifications. This is used as the Extracted Internal Standard (EIS).

5.3.1.3 13C12-6PPD-Quinone: Cambridge Isotopes CLM-11290 or equivalent. Store according to vendor specifications. This is used as sampling Recovery Standard when requested.

5.3.1.4 EIS Spike: Dilute EIS to 200 ng/mL with Acetonitrile. 100 uL of EIS Spike is added to a sample with a final extract volume of 10 mL.

5.3.1.5 NIS Spike: Dilute NIS to 20 ng/mL with Acetonitrile. 1 uL of IIS Spike is added by the LC autosampler for 10 uL of sample.

5.3.2 6PPD-Quinone Stock: Certified standard stock solutions from certified standard vendors (HPC Standards 688152, Cambridge Isotopes ULM-12288-S, or equivalent). Store according to vendor specifications.

5.3.2.1 6PPD-Quinone Intermediate Stock: Dilute 6PPD-Quinone Stock to 1000 ng/mL with Acetonitrile.

5.3.2.2 Matrix Spike: Dilute 6PPD-Quinone Stock to 200 ng/mL with Acetonitrile.

5.3.2.3 LLOQ Spike: Dilute Matrix Spike to 2.5 ng/mL with Acetonitrile.

5.3.2.4 ICAL Standards: Dilute in acetonitrile the 6PPD-Quinone Intermediate Stock, Matrix Spike, or LLOQ spike to the calibration concentrations and add EIS Spike to a final concentration of 2 ng/mL. The suggested ICAL concentrations are 0.025, 0.05, 0.5, 1, 2, 5, 10, 25, 50, and 100 ng/mL.

5.3.2.5 CCV: Use the equivalent ICAL standard. Suggested concentration is 2 ng/mL.

5.3.2.6 ICV: Prepared the same as the ICAL standard but with a different vendor. Suggested concentration is 2 ng/mL.

5.3.2.7 Instrument Sensitivity Check (ISC): The ISC is the lowest ICAL standard within the quantitation range. Currently, the concentration is at 0.05 ng/mL.

5.3.2.8 Instrument Blank: Acetonitrile with EIS at the concentration of 2 ng/mL.

5.3.3 Standard concentrations can differ from those stated in this SOP. Document all standard preparations in the standards section of the LIMS.

5.3.4 Store certified standard stocks as recommended by the vendor.

5.3.5 All intermediates, spikes, ICAL, ICV, CCV, and ISC standards are stored refrigerated. The maximum expiration is 6 months from the date of preparation or the expiration of the components whichever is shorter.

5.4 Supplies

5.4.1 SPE Cartridge: Waters Oasis HLB 6cc (200mg) SPE cartridge (WAT 106202) or Bakerbond Speedisk H2O-Philic DVB (8072-07) or Phenomenex Strata-XL 100um Polymeric Reversed Phase 100mg /6mL SPE Cartridge (8B-S043) or equivalent

5.4.2 Vacuum manifold: 12 or 24 port Supelco Visiprep or 6 port vacuum manifold & reservoir apparatus for Speedisk or equivalent.

5.4.3 Transfer tubing for HLB 6cc SPE cartridges.

5.4.4 Syringes – assorted sizes for the preparation of standards and spiking to samples.

5.4.5 2mL autosampler vials with crimp-top caps or screw-caps.

5.4.6 15 mL sample vials

5.4.7 Class A volumetric flasks of various sizes.

5.4.8 Filter Aid (optional) - Empore™ Filter Aid 400 (66897-U) or equivalent

5.4.9 Silica Cleanup Cartridges (optional) - Thermo Scientific HyperSep Silica Cartridges 100 mg (03-251-260) or equivalent

6.0 Summary of Method

6.1 This SOP describes procedures for the extraction and the qualitative and quantitative analysis of 6PPD-Quinone by triple quadrupole mass spectrometry.

6.2 This method uses reverse phase high performance liquid chromatographic, electrospray ionization with jet stream technology (ESIJT), and triple quadrupole mass spectrometric (LC-QQQ) conditions. Detection is achieved using positive ESIJT and a triple quadrupole mass spectrometer. Quantitative analysis is performed using Isotopic Dilution.

6.3 250 mL water samples are spiked with isotopically labeled 6PPD-Quinone (EIS). The necessary QC samples are also spiked with the target analyte(s) at this time. The samples are then extracted using SPE.

6.4 Interferences

6.4.1 Method interferences may be caused by contaminants in solvents, reagents, glassware and other sample processing apparatus that lead to discrete artifacts or elevated baselines in liquid chromatograms. All reagents and apparatus must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory method blanks. To minimize interference from sample matrix, this method is best utilized with samples of known matrix and interferences.

6.4.2 Raw LC-MS/MS data from all blanks, samples, and spikes must be evaluated for interferences. Determine if the source of interference is in the preparation and/or cleanup of the samples and take corrective action to eliminate the problem.

6.4.3 Cross contamination may occur when a sample containing a low concentration of analytes is analyzed immediately following a sample containing relatively high concentrations of analytes. After analysis of a sample containing high concentrations of analytes, one or more laboratory method blanks must be analyzed.

6.4.4 Matrix interference may be caused by contaminants that are present in the sample. The extent of matrix interference varies considerably from sample to sample, depending on the source sampled. Positive identifications must be confirmed by retention times, precursor ions, product ions, and product ion ratios. Samples can exhibit matrix suppression so extracting a subsample or dilution of the extract may be necessary to minimize the matrix interference.

6.5 Sample Collection, Preservation, Storage, and Holding Times

6.5.1 Water grab samples are collected in 250 mL amber glass bottles. Conventional sampling practices should be followed.

6.5.2 At this time, no preservative has been established for 6PPD-Quinone. For now, unpreserved samples are used.

6.5.3 Water samples must be stored at a temperature above freezing and up to 6°C from collection until sample preparation.

6.5.4 Water samples must be extracted within 14 days from sample collection.

6.5.5 Extracts must be stored at 0 – 6 °C and analyzed within 28 days from extraction.

6.6 Calibration and Standardization

6.6.1 Instrument Tune

6.6.1.1 Perform a check tune prior to an initial calibration to monitor the instrument status. The check tune requirements are set by the manufacturer and are noted on the check tune report.

6.6.1.2 If there are Mass Calibration results are out of criteria in the check tune report, check the tune solution and spray nozzle and/or adjust the failing tune parameter in manual tune. Perform another check tune. If the criteria is still not met, then instrument maintenance and/or a full autotune are required.

6.6.1.3 The autotune is performed at least annually or as recommended by the instrument manufacturer, whichever is more frequent.

6.6.1.4 All check tunes are accessible via the MassHunter acquisition software.

6.6.2 Initial Calibration (ICAL)

6.6.2.1 Prepare calibration standards at a minimum of six concentration levels for each analyte of interest (See Section 5.3). Seven calibration standards are needed for quadratic calibrations. The lowest standard represents analyte concentrations at or below the LOQ.

6.6.2.1.1 Initial calibrations are preformed prior to analyzing samples and are repeated as needed when calibration verification (CCV) or Instrument Sensitivity Check (ISC) is no longer within criteria or at a minimum frequency of annually.

6.6.2.2 Analyze each calibration standard using the MassHunter Software. Calculations are performed by the instrument's software. MassHunter Software has many options for calibration curves which may be used. The native unlabeled analytes are calibrated by isotope dilution where the EIS is the associated internal standard. The NIS is the associated internal standard for the EIS. See Appendix B for the internal standard associations.

6.6.2.3 All analytes must meet or exceed one of the following calibration model criteria:

6.6.2.3.1 Average Response Factor:
Minimum 6 ICAL points and %RSD ≤ 20%

Average Response Factor equation: $y = x/RF$

Where y = Instrument Target Concentration/ Instrument IS Concentration
x = Target Response/ IS Response
RF = Average Response Factor

6.6.2.3.2 Linear curve:
Minimum 6 ICAL points and %RSE ≤ 20%

Linear Equation: $y = ax + b$

Where y = Instrument Target Concentration/ Instrument IS Concentration
x = Target Response/ IS Response
a = Slope of the regression line
b = y-intercept of the regression line

6.6.2.3.3 Quadratic curve:
Minimum 7 ICAL points and %RSE ≤ 20%

Quadratic Equation: $y = ax^2 + bx + c$

Where y = Instrument Target Concentration/ Instrument IS Concentration
x = Target Response/ IS Response
a, b, c = quadratic coefficients

6.6.2.4 If the instrument sensitivity or the instrument linearity criteria for the initial calibration are not met, inspect the system for problems and take corrective actions to achieve the criteria. Instrument maintenance may need to be performed or new calibration standards may need to be prepared.

6.6.3 Initial Calibration Verification (ICV)

6.6.3.1 As of EPA Draft Method 1634 (January 2024), the Initial Calibration Verification (ICV) is not a requirement, but it is useful to confirm the standards of the primary source used for calibrations. If a secondary source is available, MEL must evaluate an ICV.

6.6.3.2 The initial calibration curve for each target analyte is verified with a standard from a source different from that used for the initial calibration. This standard must be made using stock standards prepared independently from those used for calibration. Preferably an alternate vendor is used. If an alternate vendor is not available, a different lot number from the same vendor may be used.

6.6.3.3 Analyze the ICV standard directly after calibration.

6.6.3.4 The analyte recoveries should be within 70-130% of their expected concentration. Recoveries outside of those criteria may be cause for concern. Use professional judgement to determine appropriate corrective action for ICV outlier(s).

6.6.3.5 Qualify data as appropriate based on the MEL SOP 730121 for Data Qualification.

6.6.4 Back Calculation (Residuals)

6.6.4.1 Although not explicitly required by EPA Draft Method 1634 (January 2024), back calculations are a useful tool to assess the fit of a curve by comparing the actual responses for each analyte in the standard to calculated response.

6.6.4.2 Re-calculate each ICAL concentration level using the updated calibration curve. The percent difference between the calculated concentration and the expected concentration met for each analyte at that level should not be more than ±30% or ± 50% for the lowest standard used in the curve. Higher percent differences may be cause for concern. Use professional judgement to determine appropriate corrective action for compound concentrations that do not meet the acceptance limits.

6.6.4.3 Qualify data as appropriate based on the MEL SOP 730121 for Data.

6.6.5 Instrument Sensitivity Check (ISC)

6.6.5.1 The instrument sensitivity check solution (See section 5.3) is used to check instrument sensitivity.

6.6.5.2 The signal to noise ratio must be greater than or equal to 3:1 for the quantitation ions and meet ion ratio requirements.

6.6.5.3 The recovery must be within 50-150% of the expected concentration.

6.6.6 Continuing Calibration Verification (CCV or VER).

6.6.6.1 The CCV is a mid-level calibration standard with the same concentration used during the initial calibration.

6.6.6.2 Analyze the CCV standard prior to the analysis of samples and blanks, after every 10 field samples or less, and at the end of an analytical sequence containing samples.

6.6.6.3 The recovery of the target analyte and EIS compound for the VER must be within 70 - 130%.

6.6.6.4 If a CCV does not meet quality criteria then instrument maintenance, repreparation of the standard, and/or recalibration of the instrument may be needed. Reanalyze any extracts with the failing bracketing CCVs. If the CCV failed with a high recovery and the analyte was not detected, the sample extract does not need to be reanalyzed. On a case-by-case basis, samples associated with CCV(s) not meeting acceptance limits can be reported so long as they are qualified appropriately as per the MEL SOP 730121 for Data Qualification.

7.0 Procedure

7.1 Sample Preparation:

7.1.1 Spike samples and QC samples with EIS spike and matrix spike as needed.

7.1.2 Place a SPE cartridge on the vacuum manifold for each sample and QC.

7.1.3 Filter aid (Optional)

7.1.3.1 Add about 2 grams of filter aid to the SPE cartridge. Filteraid helps prevent clogging of the SPE cartridge. If filter aid is used for a sample, all QC samples in the batch must do the same.

7.1.4 Condition the SPE cartridges by adding about 5 mL of Acetonitrile to each and allow it to flow through at a vacuum flow rate of 2.5 – 3.0 mL/minute.

7.1.5 Then condition with about 10 mL of Milli-Q water and allow it to pass through. Before the cartridge goes dry, load the sample at a vacuum flow rate of 2.5 – 3.0 mL/minute.

7.1.6 Rinse the sample bottle with about 10 mL of Milli-Q water and load the rinse through the SPE cartridge.

7.1.7 (Optional) Rinse the SPE cartridge with about 5 mL of 1:1 Methanol:Water and then 5 mL of Hexane.

7.1.8 Increase the vacuum to maximum for at least 5 minutes to dry the SPE cartridge.

7.1.9 Remove from vacuum and add a 15mL vial under each SPE cartridge to collect eluent.

7.1.10 Add 5mL of Acetonitrile to the sample bottle. Cap and shake well to extract any analytes from the inside glass surface. Add this to the top of the SPE cartridge and elute.

7.1.11 Elute with an additional 5 mL of Acetonitrile.

7.1.12 Bring to a final volume of 10 mL.

7.1.13 Cleanup (optional)

7.1.13.1 After extraction, a cleanup can be used to clean out any matrix from the sample extract before running on the instrument. If a cleanup is done for a sample, all QC samples in the batch must do the same.

7.1.13.2 Silica Gel Cartridges: Place a cleanup cartridge onto an autosampler vial. Load 1 mL of the sample extract through the cleanup cartridge. Discard the cartridge and cap the vial.

7.2 Sample Analysis:

7.2.1 Instrument run setup.

7.2.1.1 Starting the instrument.

7.2.1.1.1 If the system has been turned off, turn on the computer, mass detector, autosampler, pump and degas unit.

7.2.1.1.2 Start Triple Quadrupole (MassHunter) software. Ensure that all systems are communicating and status lights are yellow or green.

7.2.1.1.3 Load the current analysis method.

7.2.1.1.4 If needed, perform routine maintenance. See Appendix D for maintenance information.

7.2.1.2 Run a check tune if running an initial calibration.

7.2.1.2.1 Prior to running an autotune or check tune, let the pump equilibrate for approximately 20 minutes. Check background spectra in tune. Check abundance of ions in the tune. See Section 6.6.1 for more information.

7.2.1.3 Prepare the sample vials for the sequence.

7.2.1.3.1 Transfer samples, batch QC, and necessary QC standards into autosampler vials.

7.2.1.3.2 The NIS Spike standard is added by the autosampler program during the injection sequence. Fill the vial that holds the NIS spike solution with a fresh aliquot as needed.

7.2.1.3.3 Load vials for analysis onto the autosampler tray.

7.2.1.4 Setting up a Worklist.

7.2.1.4.1 Go to the Worklist tab to show the worklist spreadsheet.

7.2.1.4.2 Enter Sample name, Sample position, Comment, Method, and Data file. Other settings in the worklist can just stay at the default setting.

7.2.1.4.3 If the instrument has been idle, add at least 3 conditioning runs to the beginning of the sequence. This helps the retention times stabilize.

7.2.1.4.4 Typical ICAL sequence run:

1. If instrument has been idle, minimum 3 conditioning injections
2. Instrument Blank
3. ICAL Standards (See section 6.6.2)
4. ICV (See section 6.6.3.)

7.2.1.4.5 Typical Sample sequence run:

1. If instrument has been idle, minimum 3 conditioning injections
2. Instrument Blank
3. Instrument Sensitivity Check
4. CCV
5. Method Blank (MB)
6. OPR
7. Samples (Up to 10 injections of sample extracts, diluted extracts, laboratory duplicate extracts and MS/MSD extracts)
8. CCV
9. Instrument Blank
10. Samples (Up to 10 injections of sample extracts, diluted extracts, laboratory duplicate extracts and MS/MSD extracts)
11. CCV

7.2.1.4.6 (Recommended) At the end of the sequence, add 2 solvent rinse runs.

7.2.1.4.7 Run the Worklist.

7.2.2 Process the sample results using the MassHunter Quantitative Analysis.

7.2.2.1 Any samples outside of the criteria outlined in Section 6.6 (Calibration and Standardization) and Section 9.0 (Quality Control and Quality Assurance) may need to be rerun and reanalyzed.

7.2.2.2 Sample Dilutions

7.2.2.2.1 Dilute samples with concentrations exceeding the calibration range to bring the area of the quantitation ion to within the calibration range.

7.2.2.2.2 Since the EIS is also diluted and is used to quantify the target compound, the EIS must meet the qualitative requirements in section 7.2.3.1 and the EIS recovery limits in section 9.10.

7.2.2.2.3 If the EIS response is outside of the qualitative and recovery criteria in the dilution, the target compound cannot be calculated by isotope dilution. If additional sample is available, re-extract the sample using a smaller initial volume. If the sample cannot be re-extracted, the results must be qualified and reported as an estimated value.

7.2.2.2.4 Screening samples: If high concentrations are expected, it may be beneficial to analyze a direct injection or dilution of the sample prior to sample preparation. Depending on the result, a smaller initial volume of sample can be extracted.

7.2.3 Calculations

7.2.3.1 Qualitative Identification of Target Compounds

7.2.3.1.1 Target compound identification is made by precursor and product ions as well as retention time matching. The precursor ions are mass filtered in MS1 then they enter the collision cell where the ions collide. The ions are filtered again in MS2 and then product ions are detected. This process eliminates much interference which aides in compound identification since we are looking for compounds that begin at one mass and are then broken into certain ions with a specific ratio. Sample compound and a current laboratory-generated standard must be present and compared.

7.2.3.1.2 Using available software, search for each target compound in the established retention time window. Examine chromatograms and determine if a positive identification is present.

7.2.3.1.3 Examine baseline and peak integration to insure proper area integration. If the compound is present but not properly integrated, then manually integrate the peak. See SOP 730127 Proper Manual Peak Integration.

7.2.3.1.4 Examine transition and all product ions for confirmation ions to further validate the compound identification.

7.2.3.1.5 If there is evidence of retention time shift, use relative retention to the surrogate or internal standard along with confirming ions to validate the identification.

7.2.3.1.6 Technical Acceptance Criteria are determined by qualitative analysis of ion retention times, transition ions (precursor and product ions), chromatography, and ion abundance ratios.

7.2.3.1.7 Signal-to-Noise:

Peak responses for target analytes must be at least three times the background noise level (signal-to-noise ratio S/N ≥3:1) and the EIS and NIS response must have S/N of at least 10:1.

7.2.3.1.8 Retention Time:

The RTs for the target analyte, EIS, and NIS compounds must fall within 0.4 minutes of the predicted retention times from the midpoint standard of the ICAL or initial daily CCV, whichever was used to establish the RT window position for the analytical batch.

7.2.3.1.9 Relative Retention Time:

For all target analytes with exact corresponding isotopically labeled analogs, target analytes must elute within 0.1 minutes of the associated EIS compound.

Use professional judgment when there are retention time shifts. Document when reporting results outside of criteria including rationale.

7.2.3.1.10 Ion Abundance Ratio (IAR):

The Ion Abundance Ratio is calculated according to the equation below.

$$IAR = \frac{Area_{Q1}}{Area_{Q2}}$$

Where:

IAR = Ion Abundance Ratio
$Area_{Q1}$ = Area of Q1 (quantitation ion)
$Area_{Q2}$ = Area of Q2 (qualifier ion)

The acceptance window for the IAR of each target is 50%-150% of the IAR of the mid-point calibration standard.

7.2.3.2 Quantitative analysis of target analytes:

7.2.3.2.1 When a compound has been identified, the quantification of that compound is based on the integrated abundance from the primary product ion (also called the quantifying ion). The initial calibration (see Section 6.6.2) is used for the determination of the extract concentration.

7.2.3.2.2 Sample Dilutions

As this is an isotope dilution method, calculation of the on column concentration when a sample is diluted is taken into account by the response of the extracted internal standard. The EIS is added to the sample prior to extraction therefore, it is also diluted by the same factor as all other analytes. A separate dilution factor is not required in the calculation of the target analyte, 6PPD-Q (see equation in Section 7.2.3.2.3). The surrogate compound is calculated using the injected internal standard (IIS) and is not calculated in the same way as 6PPD-Q (see equation in Section 7.2.3.2.4)

If the area of Q1 exceeds the calibration range, dilute the sample extract to bring the concentration to within the calibration range. The EIS must meet the S/N criteria in 7.2.3.1 in the diluted extract.

If the needed dilution causes the EIS to not meet S/N criteria or become not detected, the target analyte cannot be calculated reliably in the diluted extract. If there is additional sample available, re-extract the sample with a lower initial volume. If the sample cannot be re-extracted, dilute to a level where the EIS meets the S/N criteria, and report the results as an estimate. Use professional judgment and document when reporting results outside of criteria.

7.2.3.2.3 For 6PPD-Q:

$$C_I = \frac{(Area_n)(M_{EIS})}{(Area_{EIS})(\overline{RF})}$$

Where: C_I = On column Concentration (ng/mL)
$Area_n$ = The measured area of 6PPD-Q
$Area_{EIS}$ = The measured area for the EIS
M_{EIS} = The Concentration of the EIS added (ng/mL)
$\overline{RF}$ = Average response factor

7.2.3.2.4 And for the SS analyte:

$$C_I = \frac{(Area_{SS})(M_{IIS})}{(Area_{IIS})\overline{(RF_s)}}$$

Where: C_I = Final Concentration (ng/mL)
$Area_{SS}$ = The measured area of D5-6PPD-Q
$Area_{IIS}$ = The measured area of 13C6-6PPD-Q
M_{IIS} = The concentration of the IIS added (ng/mL)
$\overline{RF_s}$ = Average response factor

7.2.3.3 Calculate the concentration of each identified analyte in the sample as follows:

$$C_F = \frac{C_I(v_F)(D)}{v_I}$$

Where: C_F = Final Concentration (ng/L)
C_I = On Column Concentration (ng/mL)
V_F = Final Volume of Extract (mL)
D = Dilution Factor (only used for surrogate)
V_I = Initial Volume of Sample (mL)

Results are reported as nanograms/liter (ng/L).

7.2.3.4 Ongoing Precision and Recovery (OPR) recoveries are calculated as follows:

$$Recovery(\%) = \frac{MCSS}{SCA} \times 100$$

Where: MCSS = Measured Concentration of Spiked Sample

SCA = Spike Concentration Added

7.2.3.5 If an Ongoing Precision and Recovery (OPR) and an Ongoing Precision and Recovery Duplicate pair was analyzed, calculate the Relative Percent Difference (RPD) of each compound as follows:

$$RPD=\left[\frac{|OPR-OPRD|}{(OPR+OPRD)/2}\right]\times 100$$

Where: OPR = OPR Recovery

OPRD = OPR Duplicate Recovery

7.2.3.6 Matrix Spike (MS) recoveries are calculated as follows:

$$MSR=\left[\frac{MCSS-MSSC}{SCA}\right]\times 100$$

Where: MCSS = Measured Concentration of Spiked Sample

MSSC = Measured Source Sample Concentration

SCA = Spike Concentration Added

MSR% = Matrix Spike Recovery %

7.2.3.7 If a Matrix Spike and Matrix Spike Duplicate (MS/MSD) pair was analyzed, calculate the RPD of each compound as follows:

$$RPD=\left[\frac{|MSR-MSDR|}{(MSR+MSDR)/2}\right]\times 100$$

Where: MSR = Matrix Spike Recovery

MSDR = Matrix Spike Duplicate Recovery

8.0 Records Management

8.1 Retain raw data for 7 years following reporting. The data PDF reports are stored in Element. Raw data are also stored on the instrument computer or in a designated area for 7 years.

8.2 Instrument and/or sample preparation logbooks are kept next to the instrument or with the Chemist performing the analysis.

8.2.1 When the logbooks are full, they are given to the MEL QA Coordinator for filing and secure storage.

8.2.2 Logbooks used to document instrument maintenance or routine documentation of a single piece of equipment are retained for 10 years after the retirement of the instrument/equipment.

8.2.3 Logbooks used to document procedures, such as preparation/extraction, preservation, etc. not tied to specific to equipment, or that are used to document quality control of more than one piece of equipment, are retained for 10 years after submission to QAC for secure storage.

8.3 The LCMSMS Data Review Checklist can be found in MEL's SharePoint page under Organics – Documents – Data Review. The checklist indicates what reports and data must be included with the work order package.

8.4 MassHunter generates the following reports: Sequence Logs, Tune Reports, ICAL Reports, and Quantitation Reports.

8.5 Element generates the following reports: Sample Preparation Batch, Sequence Report, Review Reports, and Final Reports.

8.6 If necessary, the Corrective Action Form (CAF) can be found in MEL's SharePoint page under Organics – Forms.

9.0 Quality Control and Quality Assurance

9.1 Refer to client's QAPP for special QA/QC protocols.

9.2 Samples are qualified following data qualification SOP 730121 guidelines.

9.3 Non-extracted internal standard (NIS):

9.3.1 Each sample run is spiked with the NIS to a concentration of 2 ng/mL by the instrument.

9.3.2 The NIS peak area of the QC and field samples must be within 50-200% compared to the average NIS area of the initial calibration.

9.3.3 Reanalysis is necessary for any sample outside of NIS criteria. If reanalysis confirms this variance in signal, all the analytes associated with that internal standard must be qualified following data qualification SOP 730121 guidelines.

9.3.4 Sample Dilution: Instead of reanalysis at the original LLOQ, reanalysis of the sample at a dilution may minimize the NIS failure by lessening matrix interference. Use professional judgment to decide the best way to report the results.

9.4 Extracted Internal Standard (EIS):

9.4.1 The EIS is added during preparation of the samples and calibration standards.

9.4.2 EIS recoveries for field samples must fall within the recovery acceptance criteria of 25–200%.

9.5 Instrument Blank:

9.5.1 The instrument blank is analyzed to ensure that no instrument contamination has occurred.

9.5.2 The instrument blank is analyzed prior to the start of the analytical sequence and after any time a sample with high concentrations is expected.

9.5.3 If detections in the instrument blank are found, use professional judgement to determine if instrument maintenance and/or reanalysis of the affected samples is necessary.

9.6 Method Blank:

9.6.1 A Method Blank (MB) must be prepared with each extraction batch of 20 or fewer samples.

9.6.2 The blanks must be free from contamination at a concentration at or below ½ the LLOQ.

9.6.2.1 If the MB fails to meet quality criteria, the analyst determines whether to qualify the data, reanalyze, or re-extract the samples depending on severity of contamination and project objectives. At a minimum, the reanalysis includes the MB and the affected samples.

9.6.2.2 If low reporting limits are not required, the RL may be raised, per client approval.

9.6.2.3 On a case-by-case basis, per client or supervisor approval, samples associated with a MB not meeting acceptance limits can be reported so long as they are addressed in the case narrative and qualified following data qualification SOP 730121 guidelines.

9.7 Laboratory Control Sample (LCS) or Ongoing Precision and Recovery (OPR):

9.7.1 One Ongoing Precision and Recovery (OPR) must be prepared with each extraction batch of 20 or fewer samples.

9.7.2 The OPR recoveries must fall within 70%-130% recovery until statistical control charting the limits.

9.7.3 As of EPA Draft Method 1634 (January 2024), laboratory spike duplicates are not required. If a duplicate was performed, the duplicate RPD should be less than or equal to 40%.

9.7.4 OPR recoveries outside criteria are typically reanalyzed to confirm results. The associated samples may need to be re-extracted if hold time and extra sample volume permits.

9.7.5 On a case-by-case basis, per client or supervisor approval, samples associated with an OPR not meeting acceptance limits can be reported so long as they are addressed in the case narrative and qualified following data qualification SOP 730121 guidelines.

9.8 Matrix Spike/Matrix Spike Duplicate (MS/MSD):

9.8.1 If requested by the client, Matrix Spike Sample and Matrix Spike Sample Duplicate (MS/MSD) are prepared with an extraction batch of 20 or fewer samples.

9.8.2 MS/MSDs generally are not required for isotope dilution methods because any deleterious effects of the matrix are generally evident in the recoveries of the labeled compounds spiked into every sample.

9.8.3 The MS/MSD recoveries should fall within 50%-150% recovery until statistical control charting the limits.

9.8.4 The duplicate RPD should be less than or equal to 40%.

9.8.5 MS/MSD samples are typically not re-prepared or re-analyzed unless obvious preparation or analysis errors occurred or the results are grossly outside criteria.

9.8.6 For results outside of the acceptance limit, qualify the source sample analytes as estimates following data qualification SOP 730121 guidelines. All other anomalies are dealt with on a case-by-case basis and referred to the supervisor.

9.9 Sample Duplicate:

9.9.1 A DUP is analyzed if requested by the client.

9.9.2 The duplicate RPD should be less than or equal to 40%.

9.9.3 DUP samples are typically not re-prepared or re-analyzed unless obvious preparation or analysis errors occurred.

9.9.4 If the RPD fails due to heterogeneity or matrix interference, qualify the failing analytes in the source sample following data qualification SOP 730121 guidelines. All other anomalies are dealt with on a case-by-case basis and referred to the supervisor.

9.10 Surrogates:

9.10.1 The EIS is used as the surrogate. The recovery limits are 25-200%.

9.11 Investigate samples not meeting control limits to determine the root cause of QC failure(s) by checking calculation errors, standard solution degradation, contamination, and instrument performance. If applicable, make the necessary adjustments and reanalyze the sample. If the limits are met, report results from the reanalyzed sample. If the limits are still not met, re-extract if hold time and extra sample volume permits; otherwise, qualify that sample data following data qualification SOP 730121 guidelines.

9.12 Lower Level of Quantitation:

9.12.1 LLOQs are analyzed annually.

9.12.2 See SOP 770044 Method Detection Limits and Lower Limits of Quantitation/Reporting Limits.

9.13 Method Detection Limits

9.13.1 Perform an MDL study for all projects supporting the Clean Water Act or if needed for client specific projects as stated in its QAPP.

9.13.2 See SOP 770044: Method Detection Limits and Lower Limits of Quantitation/Reporting Limits.

9.14 Initial Demonstration of Capability (IDC) or Initial precision and recovery (IPR)

9.14.1 See SOP: 770032 Personnel Training.

9.14.2 IDCs are performed when:

9.14.2.1 There are new personnel responsible for analysis or sample preparation.

9.14.2.2 There is a major change in hardware.

9.14.2.3 There is a major change in sample preparation.

9.14.2.4 There is a major change to the instrument method.

9.14.2.5 New analytes are added to the method.

9.14.3 Blind Sample

9.14.3.1 Performed annually.

9.14.3.2 Another chemist (not the primary chemist for the analysis) prepares an unknown spike sample and sends the concentration information to the QAC.

9.14.3.3 The primary chemist analyzes this spiked sample.

9.14.3.4 The blind sample measured concentration must be within LCS control limits.

9.15 Document the preparation of standards in Element standard preparation module.

9.16 Document the preparation of samples in Element and the preparation logbook.

9.17 Document all instrument problems in the instrument logbook.

9.18 Print and store the sequence in the instrument logbook.

10.0 Safety

10.1 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.

10.2 The analysts must be familiar with the location and proper use of the fume hoods, eye washes, safety showers, and fire extinguishers. In addition, the analysts must wear protective clothing at all times, including safety glasses, goggles, or a face shield.

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

10.4 Work with solvents or chemicals may be performed only when at least one other person is in the area.

10.5 Follow all safety guidelines outlined in the Laboratory Health and Safety Manual and Chemical Hygiene Plan.

10.6 Waste Management/Pollution Prevention

10.6.1 Dispose of laboratory-generated waste and waste sample in accordance with the Manchester Laboratory Dangerous Waste Disposal Manual.

11.0 References

11.1 EPA Draft Method 1634: Determination of 6PPD-Quinone in Aqueous Matrices Using Liquid Chromatography with Tandem Mass Spectrometry (LC/MS/MS), January 2024

11.2 EPA SW-846 Update IV Method 8000D: Determinative Chromatographic Separations, Revision 5 March 2018

11.3 40 CFR Part 136, Appendix B, "Definition and Procedure for the Determination of Method Detection Limit", Revision 2, 8/28/17

11.4 40 CFR Part 136.6: Method modifications and analytical requirements.

11.5 40 CFR Part 136.7: Quality assurance and quality control.

11.6 Tian, et al. A Ubiquitous Tire Rubber–Derived Chemical Induces Acute Mortality in Coho Salmon. Science 2021, 371(6525), 185–189.

11.7 Quantitation of Toxic Tire Degradant 6PPD-Quinone in Surface Water, Agilent Technologies, Inc. 2021, 5994-3754EN

11.8 Agilent 6400Series QQQ LC/MS Techniques and Operation Course Number R1893A Volume I Student Manual, Data Acquisition B.02.01; Qual B.2 SP3; Quant B.03.01. 2009 Agilent Technologies, Inc.

11.9 Maintaining Your Agilent LC and LC/MS Systems. Agilent.

11.10 Manchester Environmental Laboratory Quality Assurance Manual, Washington State Department of Ecology.

11.11 Chemical Hygiene Plan, US EPA Region 10 Laboratory.

11.12 Dangerous Waste Disposal Manual, US EPA Region 10 Laboratory and Washington State Dept. of Ecology.

11.13 Laboratory Health and Safety Manual for US EPA Region 10 Laboratory and Washington Department of Ecology Laboratory.

11.14 MEL SOP 730121: Data Qualification of Organic Sample Results.

11.15 MEL SOP 730127: Proper Manual Peak Integration

11.16 MEL SOP 770044: Method Detection Limits and Lower Limits of Quantitation/Reporting Limits

11.17 MEL SOP 770032 SOP for Personnel Training

Appendix A: Compound List and Transitions

Table A01

Analyte	CAS	Quantitation Transition (Q1)	Qualifier Transition (Q2)	Ion Polarity
6PPD-quinone	2754428-18-5	299.1 → 215.1	299.1 → 241.1	Positive
13C6-6PPD-Quinone (EIS/Surrogate)	NULL	305.1 → 221.1	305.1 → 247.1	Positive
D5-6PPD-quinone (NIS)	NULL	304.1 → 220.1	304.1 → 246.1	Positive
13C12-6PPD-Quinone (Recovery Standard)	NULL	311.1 → 253.1	311.1 → 227.1	Positive

Note 1: This table has the current compound list for this method. Depending on demand, compounds may be added or removed. Additional compounds require further requirements (see Section 9).
Note 2: This table has the current transitions used for this analysis. Alternate transitions may be used as long as they are consistent with the ICAL used for calculations.

Appendix B: Retention Times and IS Associations

Table B01

Analyte	Retention Time	Associated IS
6PPD-quinone	6.27	13C6-6PPD-quinone (EIS)
13C6-6PPD-Quinone (EIS/Surrogate)	6.27	D5-6PPD-quinone (NIS)
D5-6PPD-quinone (NIS)	6.27	NA
13C12-6PPD-Quinone	6.27	13C6-6PPD-quinone (EIS)

Note 1: Retention Times are approximate and can change depending on instrument conditions.

Appendix C1: Instrument Method (6460 Model)

Method Name: 6PPDQ_2022A.m

Method Path: C:\MassHunter\methods\CURRENT METHODS\6PPDQ_2022A.m

MS QQQ Mass Spectrometer Model G6460A Settings:

Table C1-01: MS Settings

Parameter	Setting
Ion Source	AJS ESI
Stop Mode	No Limit/As Pump
Time Filter	On
LC->Waste Pre Row	N/A
Tune File	C:\MassHunter\Tune\QQQ\G6460A\atunes.TUNE.XML
Stop Time (min)	No limit
Time Filter Width (min)	0.05
LC->Waste Post Row	N/A

Table C1-02: MS Time Segments

Index	Start Time (min)	Scan Type	Ion Mode	Div Valve	Delta EMV (+)	Store	Cycle Time (ms)	Triggered?	MRM Repeats
1	0.4	Dynamic MRM	ESI+ Agilent Jet Stream	To MS	400	Yes	500	No	3

Table C1-03: MS Scan Segments

Cpd Name	Prec Ion	MS1 Res	Prod Ion	MS2 Res	Frag (V)	CE (V)	Cell Acc (V)	Ret Time (min)	Ret Window	Polarity
6PPD-quinone	299.1	Unit/Enh (6490)	256.1	Unit/Enh (6490)	140	20	4	7.3	3	Positive
6PPD-quinone	299.1	Unit/Enh (6490)	241.1	Unit/Enh (6490)	105	32	4	7.3	3	Positive
6PPD-quinone	299.1	Unit/Enh (6490)	215.1	Unit/Enh (6490)	105	16	4	7.3	3	Positive
6PPD-quinone	299.1	Unit/Enh (6490)	187.1	Unit/Enh (6490)	105	32	4	7.3	3	Positive
6PPD-quinone	299.1	Unit/Enh (6490)	170.1	Unit/Enh (6490)	120	30	4	7.3	3	Positive
D5-6PPDQuinone	304.1	Unit/Enh (6490)	246.1	Unit/Enh (6490)	110	36	4	7.3	3	Positive
D5-6PPDQuinone	304.1	Unit/Enh (6490)	220.1	Unit/Enh (6490)	110	20	4	7.3	3	Positive
13C6-6PPDQuinone	305.1	Unit/Enh (6490)	247.1	Unit/Enh (6490)	110	36	4	7.3	3	Positive
13C6-6PPDQuinone	305.1	Unit/Enh (6490)	221.1	Unit/Enh (6490)	110	20	4	7.3	3	Positive

Table C1-04: MS Scan Parameters

Data Stg	Threshold
Centroid	0

Table C1-05: MS Source Parameters

Parameter	Value (+)	Value (-)
Gas Temp (°C)	300	300
Gas Flow (l/min)	10	10
Nebulizer (psi)	40	40
Sheath Gas Heater	375	375
Sheath Gas Flow	11	11
Capillary (V)	2500	0
V Charging	0	0

Table C1-06: MS Chromatograms

Chrom Type	Label	Offset	Y-Range
TIC	TIC	0	1500000

Sampler Model G1329B:

Table C1-07: Sampler Settings

Parameter	Setting
Auxiliary: Draw Speed	200 µL/min
Auxiliary: Eject Speed	100 µL/min
Auxiliary: Draw Position Offset	5.0 mm
Injection Mode	Standard injection
Injection Volume	5.00 µL
Enable Overlapped Injection	No
Stoptime Mode	As pump/No limit
Posttime Mode	Off
Pretreatment Step 1: Wash	Wash needle in location "Vial 92" 1 times
Pretreatment Step 2: Draw	Draw 1 µL from location "Vial 91" with default speed using default offset
Pretreatment Step 3: Wash	Wash needle in location "Vial 92" 1 times
Pretreatment Step 4: Draw	Draw 10 µL from sample with default speed using default offset
Pretreatment Step 5: Inject	Inject

Note 1: A vial of Methanol is in location “Vial 92” of the sample tray.
Note 2: A vial of the IIS solution is in location “Vial 91” of the sample tray.

Table C1-08: Column Comp. Settings

Parameter	Setting
Valve Position	Position 1 (Port 1 -> 2)
Left Temperature Control Mode	Temperature Set
Left Temperature	40.0 °C
Enable Analysis Left Temperature On	Yes
Enable Analysis Left Temperature Value	0.8 °C
Right Temperature Control Mode	Combined
Enable Analysis Right Temperature On	Yes
Enable Analysis Right Temperature Value	0.8 °C
Stop Time Mode	As pump/injector
Post Time Mode	Off

Binary Pump Model G1312B:

Table C1-09: Binary Pump Settings

Parameter	Setting
Flow	0.400 mL/min
Use Solvent Types	No
Low Pressure Limit	0.00 bar
High Pressure Limit	590.00 bar
Maximum Flow Gradient	100.000 mL/min²
Automatic Stroke Calculation A	Yes
Automatic Stroke Calculation B	Yes
Compressibility Mode A	Compressibility Value Set
Compressibility A	50 10e-6/bar
Compressibility Mode B	Compressibility Value Set
Compressibility B	115 10e-6/bar
Stop Time Mode	Time set
Stop Time	10.5 min
Post Time Mode	Time set
Post Time	4.00 min

Table C1-10: Binary Pump Solvent Composition

Solvent Composition	Channel	Name 1	Selected	Used	Percent
1	A	H2O (0.1% formic)	Ch. 1	Yes	90.0 %
2	B	ACN (0.1% formic)	Ch. 1	Yes	10.0 %

Table C1-11: Binary Pump Timetable

Timetable	Time	A	B	Flow	Pressure
1	0.50 min	90.0 %	10.0 %	0.400 mL/min	590.00 bar
2	5.00 min	15.0 %	85.0 %	0.400 mL/min	590.00 bar
3	10.00 min	0.0 %	100.0 %	0.400 mL/min	590.00 bar
4	10.50 min	0.0 %	100.0 %	0.400 mL/min	590.00 bar

Appendix C2: Instrument Method (Ultivo Model)

Method Name: 2024 6PPDQ Acquisition 13C6 Double Vol.m
Method Path: C:\MassHunter\Methods\10.0\Acquisition\2024 6PPDQ Acquisition 13C6 Double Vol.m

Table C2-01: Multisampler G7167B Settings

Parameter	Setting
Sampling Speed	
Draw Speed	100.0 µL/min
Eject Speed	400.0 µL/min
Wait Time After Drawing	1.2 s
Injection	
Needle Wash Mode	Standard Wash
Injection Volume	5.00 µL
Standard Needle Wash	
Needle Wash Mode	Flush Port
Duration	3 s
High Throughput	
Injection Valve to Bypass for Delay Volume Reduction	No
Sample Flush-Out Factor	5.0
Overlap Injection Enabled	No
Needle Height Position	
Draw Position Offset	0.0 mm
Use Vial/Well Bottom Sensing	Yes
Stoptime Mode	As Pump/No Limit
Posttime Mode	Off
Pretreatment	
Wash	Wash needle in "Vial 1" 1 times
Draw	Draw 1.00 µL from location "Vial 4" with default speed using default offset
Wash	Wash needle in flushport with "S1" for 3 s
Draw	Draw 10.00 µL from sample with default speed using default offset
Inject	Inject

Table C2-02: Binary Pump G7120A Settings

Parameter	Setting
Flow	0.300 mL/min
Use Solvent Types	Yes
Stroke Mode	Synchronized
Low Pressure Limit	0.00 bar
High Pressure Limit	590.00 bar
Max. Flow Ramp Up	100.000 mL/min²
Max. Flow Ramp Down	100.000 mL/min²
Expected Mixer	No check
Automatic Stroke Calculation A	Yes
Stoptime Mode	Time set
Stoptime	7.50 min
Posttime Mode	Time set
Posttime	2.50 min

Table C2-03: Binary Pump Solvent Composition

Solvent Composition	Channel	Name	Percent
1	A	0.1% Formic Acid/Water	95.00 %
2	B	0.1% Formic Acid/ACN	5.00 %

Table C2-04: Binary Pump Timetable

Timetable	Time	A	B	Flow	Pressure
1	Start. Cond. min	95.00 %	5.00 %	0.300 mL/min	590.00 bar
2	1.00 min	95.00 %	5.00 %	0.300 mL/min	590.00 bar
3	4.00 min	50.00 %	50.00 %	0.300 mL/min	590.00 bar
4	7.00 min	0.00 %	100.00 %	0.300 mL/min	590.00 bar
5	8.00 min	0.00 %	100.00 %	0.300 mL/min	590.00 bar

Table C2-05: Column Comp. G7116B Settings

Parameter	Setting
Left Temperature Control	
Temperature Control Mode	Temperature Set
Temperature	40.0 °C
Enable Analysis Left Temperature	
Enable Analysis Left Temperature On	Yes
Enable Analysis Left Temperature Value	0.8 °C
Left Temp. Equilibration Time	0.0 min
Right temperature Control Mode	Combined
Enable Analysis Right Temperature	
Enable Analysis Right Temperature On	Yes
Enable Analysis Right Temperature Value	0.8 °C
Right Temp. Equilibration Time	0.0 min
Enforce column for run enabled	No
Stoptime Mode	As Pump/Injector
Posttime Mode	Off
Timetable	
Ready when front door open	Yes
Position Switch After Run	Do not switch

Table C2-06: QQQ Mass Spectrometer Ultivo Settings

Parameter	Setting
General	
Tune File	atunes.tune
Ion Source	AJS ESI
Time Filter Enabled	On
Time filter window	0.05 min
Stop Mode	By Pump Time
Current Time Segment	
Start Time (min)	0
Scan Type	dMRM

Table C2-07: QQQ Mass Spectrometer Time Segment

Start Time (min)	Scan Type	Ion Mode	Cycle Time (ms)
0	dMRM	AJS ESI	500

Table C2-08: QQQ Mass Spectrometer dMRM Settings

Compound name	Precursor (m/z)	MS1 res	Product (m/z)	MS2 res	RT (min)	RT Window (min)	Fragmentor (V)	CE (V)	Polarity
13C12-6PPD-Quinone	311.1	Unit	268.1	Unit	6.2	3	140	23	+
13C12-6PPD-Quinone	311.1	Unit	253.1	Unit	6.2	3	110	32	+
13C12-6PPD-Quinone	311.1	Unit	227.1	Unit	6.2	3	110	19	+
13C6-6PPD-Quinone	305.1	Unit	247.1	Unit	6.2	3	110	33	+
13C6-6PPD-Quinone	305.1	Unit	221.1	Unit	6.2	3	110	20	+
D5-6PPD-Quinone	304.1	Unit	246.1	Unit	6.2	3	110	33	+
D5-6PPD-Quinone	304.1	Unit	220.1	Unit	6.2	3	110	19	+
6PPD-Quinone	299.1	Unit	256.1	Unit	6.2	3	140	23	+
6PPD-Quinone	299.1	Unit	241.1	Unit	6.2	3	105	33	+
6PPD-Quinone	299.1	Unit	215.1	Unit	6.2	3	105	18	+
6PPD-Quinone	299.1	Unit	187.1	Unit	6.2	3	105	29	+
6PPD-Quinone	299.1	Unit	170.1	Unit	6.2	3	120	38	+

Table C2-09: QQQ Mass Spectrometer Source Settings

Parameter	Positive Value	Negative Value
Gas Temperature (°C)	300	300
Gas Flow (L/min)	10	10
Nebulizer (psi)	40	40
Sheath Gas Temperature (°C)	375	375
Sheath Gas Flow (L/min)	11	11
Capillary Voltage (V)	2500	0
Nozzle Voltage (V)	0	0

Table C2-10: QQQ Mass Spectrometer Timetable Settings

Start Time (min)	Timetable Type	Timetable Value
0 min	Diverter Valve	To MS
8.5 min	Diverter Valve	To Waste

Appendix D: Routine Maintenance

Routine Maintenance Schedule:

Daily Maintenance:
1. Change the wash solvents.
2. Replace NIS vial.
3. Check solvent eluent levels.
4. Check seal wash and needle wash levels.
5. Check column pressure. If it has significantly changed for no reason, reload the method, check for leaks, line kinks, pump bypass valve closure, and solvent eluent levels.

Weekly:
1. Check and drain rough pump reservoir mist filter (Model 6460 only).
2. Run a check tune.
3. Check LC waste buckets and empty as needed.

Monitor:
1. Rough Vac number: (1.62 torr is normal)
2. High Vac number (2.6 to 2.8 X 10^{-5} torr is normal)

As Required:
1. Clean the source and capillary inlet:
   a. If instrument has been on, then set to standby, turn source gas and sheath gas to 0, and cool source before cleaning.
   b. Open ESIJT source door cover, rinse and wipe down interior of the spray chamber with isopropyl alcohol or methanol.
   c. If several analytes lose sensitivity, check capillary cover for discolor, polish the capillary cover with aluminum oxide power and then sonicate in water or a mixture of water and acetonitrile or methanol or isopropyl alcohol.

2. Solvent Eluents:
   a. If necessary, Refill or Change the eluent.
   b. Prime the pumps when eluent is refilled, changed, or the system has been idle.
      i. Open the pump bypass valve and increase flow.
      ii. Increase the % of the solvent bottle being primed. Allow the solvent to flow until no bubbles can be seen going through the lines.
      iii. Decrease flow and close valve after pump is primed.

3. Reboot PC.

4. Check Software Center for computer updates.