DEPARTMENT OF ECOLOGY
State of Washington

Standard Operating Procedure MEL730136, Version 1.2

Extraction and Analysis of 6PPD-Quinone

Approved or Recertified 06/03/2023

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 will 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

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.

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	Element	MEL's Laboratory Information Management System (LIMS)
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	COD or R^2	Coefficient of Determination
3.1.12	SS	Surrogate Standard
3.1.13	EIS	Extracted Internal Standard
3.1.14	IIS	Injected Internal Standard
3.1.15	LC/HPLC	High Performance Liquid Chromatograph
3.1.16	MS/MS	Triple Quadrupole Mass Spectrometer
3.1.17	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, 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 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.

3.2.7 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.

3.2.8 Laboratory Control Sample Duplicate (LCSD): An additional replicate of the LCS following the sample preparation and analytical testing as the original LCS.

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

3.2.10 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).

3.2.11 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.

3.2.12 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.

3.2.13 Method blank (MB): 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.

3.2.14 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.

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

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

3.2.17 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.

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

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

3.2.20 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 6460A/1260 HPLC system capable of performing gradient adjustments at a constant flow rate or equivalent.

5.1.3 Agilent model 6460 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).

5.2 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 Internal Standards:

5.3.1.1 D5-6PPD-Quinone: HPC Standards 688151 or equivalent. Store according to vendor specifications.

5.3.1.2 13C6-6PPD-Quinone: Cambridge Isotopes CLM-12293-S or equivalent. Store according to vendor specifications.

5.3.1.3 Note: D5-6PPD-Quinone and 13C6-6PPD-Quinone can be used as either the EIS or IIS as long as it is consistent with the preparation batch and the instrument calibration. Currently D5-6PPD-Quinone is used as the EIS and 13C6-6PPD-Quinone is used as the IIS.

5.3.1.4 EIS/ SS 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 IIS Spike: Dilute IIS 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, 0.1, 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.

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

5.3.4 Store certified standard stocks as recommended by the vendor.

5.3.5 All intermediates, spikes, ICAL, ICV, and CCV standards are stored refrigerated. The maximum expiration is one year from the date of preparation provided none of the components are expired.

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 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.

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 should be analyzed.

6.4.4 Matrix interference may be caused by contaminants that are present in the sample. The extent of matrix interference will vary 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 Grab samples are collected in 250 mL 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 will be used.

6.5.3 Samples must be stored at a temperature above freezing and up to 6°C from collection until analysis.

6.5.4 No hold time has been established for this analyte. For now, a 28 day hold time for samples will be used.

6.5.5 The extract hold time will be 40 days after 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 more than 10 parameters out of spec or MS2 abundance for 2122 ion is less than 15000, check the tune solution and spray nozzle and/or adjust the failing tune parameter in manual tune. Perform another check tune. If this one fails, then instrument maintenance and/or a full autotune are required.

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

6.6.2 ICAL

6.6.2.1 Prepare calibration standards at a minimum of six concentration levels for each analyte of interest. The lowest standard represents analyte concentrations at or below the LLOQ.

6.6.2.1.1 Initial calibrations are preformed prior to analyzing samples and are repeated as needed when calibration verification 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.

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 5 ICAL points and %RSD ≤ 20%

Average RF 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 5 ICAL points and $R^2 \geq 0.99$;

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 6 ICAL points and $R^2 \geq 0.99$

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 Most curve fitting programs will use some form of least squares minimization to adjust the coefficients of the polynomial (a, b, and c, above) to obtain the polynomial that best fits the data. The "goodness of fit" of the polynomial equation is evaluated by calculating the coefficient of determination (COD). Under ideal conditions, with a "perfect" fit of the model to the data, the coefficient of the determination will equal 1.0. In order to be an acceptable non-linear calibration, the COD must be greater than or equal to 0.99. (See SW-846 method 8000D section 11.5.3.2).

6.6.2.4.1 If data of lesser quality will satisfy project-specific data needs, then less stringent criteria may be employed, provided that they are documented and approved in a project-specific QAPP.

6.6.3 Initial Calibration Verification (ICV).

6.6.3.1 The initial calibration curve for each target analyte must be checked immediately 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.

6.6.3.2 Analyze the ICV standard directly after calibration. The ICV is used to verify the stock after every new calibration curve that is analyzed.

6.6.3.3 The analyte recoveries should be within +/- 30% of their expected concentration. If the ICV does not meet quality criteria, the instrument may be recalibrated. If the ICV failed due to problems other than calibration, remake the standard and reanalyze. If the ICV passes, continue the sequence. If it fails, abort the sequence, determine the problem, and recalibrate the instrument. On a case by case basis, per client and supervisor approval, samples associated with an ICV not meeting acceptance limits can be reported so long as they are addressed in the case narrative and qualified as estimates.

6.6.4 Back Calculation (Residuals)

6.6.4.1 Re-calculate each ICAL concentration level using the updated calibration curve. The percent difference between the calculated concentration and the expected concentration for each analyte at that level should not be more than 30%; except for the lowest standard used in the curve, where analytes are allowed to be within 50%. If these requirements are not met, the ICAL for those analytes fails and should be reanalyzed. If the ICAL cannot be reanalyzed, all results for any analyte with a high percent difference must be qualified as estimated. If an analyte fails with a high percent difference, any detections will be qualified as estimated. If the low standard does not meet these criteria and low reporting limits are not required, the reporting limit may be raised to that of the next lowest standard which does meet the criteria.

6.6.5 Continuing Calibration Verification.

6.6.5.1 Analyze a CCV standard at a minimum of every 12 hours. The CCV analyzed is a mid level standard and should be prepared from the same source as calibration standards. The acceptance range is +/- 30%. If a CCV does not meet quality criteria, recalibrate the instrument and reanalyze the samples. On a case by case basis, samples associated with a CCV not meeting acceptance limits can be reported so long as they are qualified as estimates.

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 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.

7.1.4 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.

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

7.1.6 Rinse the SPE cartridge with about 5 mL of 1:1 Methanol:Water and then 5 mL of Hexane.

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

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

7.1.9 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.

7.1.10 Elute with an additional 5 mL of Acetonitrile.

7.1.11 Bring to a final volume of 10 mL.

7.2 Sample Analysis:

7.2.1 Instrument run setup.

7.2.1.1 Start 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 IIS Spike standard is added by the autosampler program during the injection sequence. Fill the vial that holds the IIS spike solution with a fresh aliquot each day.

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:

If instrument has been idle, minumum 3 conditioning injections
ICAL Standards – minimum of 5 standards for linear calibration and minimum 6 standards for quadratic calibration. (See section 5.3.2.4 for suggested concentrations.)
ICV (See section 5.3.2.6 for suggested concentration.)

7.2.1.4.5 Typical Sample sequence run:

If instrument has been idle, minumum 3 conditioning injections
CCV (See Section 5.3.2.5 for suggested concentration.)
MB
LCS
LCSD
Samples (up to 12 hours from CCV run)

7.2.1.4.6 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 Dilute samples with concentrations exceeding the linear range to approximately the middle of the curve and reanalyze.

7.2.2.3 Screening samples: Because the targets are calculated with an EIS, it may be necessary to prepare samples with a smaller initial volume for the analyte to be within calibration range without diluting out the EIS. If high concentrations are expected, it may be necessary to screen a dilution of the sample prior to sample preparation.

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 The relative retention times (RRTs) must be within ±0.03 RRT units of the standard RRT. Use professional judgment when there is a question if 0.03 RRT units may be too broad or too narrow. Document when reporting results outside of criteria including rationale.

7.2.3.1.8 Verify the presence of product ions and check their corresponding ratios of the analyte in the sample. Compare product ion ratios in samples against a current laboratory-generated standard (i.e., the ion ratios from the associated calibration standard). The ion ratio acceptance criteria for this method are set at +/- 30%. The relative response ratio is calculated by dividing the qualifier ion area by the quantifier ion area.

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 will be 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 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)

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 Laboratory Control Sample (LCS) recoveries are calculated as follows:

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

Where: MCSS = Measured Concentration of Spiked Sample

SCA = Spike Concentration Added

7.2.3.5 If a Laboratory Control Sample and Laboratory Control Sample Duplicate (LCS/LCSD) pair was analyzed, calculate the Relative Percent Difference (RPD) of each compound as follows:

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

Where: LCS = Laboratory Control Sample Recovery

LCSD = Laboratory Control Sample 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 lists what reports and data should 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 Internal Standards:

9.3.1 Each sample run is spiked with the IIS to a concentration of 2 ng/mL by the instrument. The EIS is added during preparation of the samples and calibration standards.

9.3.2 CCV: Reanalysis is necessary for any CCV standard in which the IIS peak area varies by more than +/- 50% from the IIS area obtained during the initial calibration.

9.3.3 Samples: Reanalysis is necessary for any sample in which the IIS peak area varies by more than +/- 50% from the IIS area of the associated CCV standard. If reanalysis confirms this variance in signal, all the analytes associated with that internal standard must be qualified following data qualification SOP 730121 guidelines.

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

9.4 Method Blank:

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

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

9.4.2.1 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.

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

9.4.2.3 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.

9.5 Laboratory Control Sample:

9.5.1 Laboratory Control Sample and Laboratory Control Sample Duplicate (LCS/LCSD) must be prepared with each extraction batch of 20 or fewer samples.

9.5.2 The LCS/LCSD recoveries should fall within laboratory control limits which are based on statistical control charts. New analytes are set at 50%-150% recovery until control charting the limits are possible.

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

9.5.4 LCS 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.

9.5.5 On a case by case basis, per client or supervisor approval, samples associated with an LCS 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.

9.6 Matrix Spike:

9.6.1 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.

9.6.2 The MS/MSD recoveries should fall within laboratory control limits which are based on statistical control charts. New analytes are set at 50%-150% recovery until control charting the limits are possible.

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

9.6.4 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.

9.6.5 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.

9.7 Sample Duplicate:

9.7.1 A DUP is analyzed if requested by the client.

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

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

9.7.4 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.

9.8 Surrogates:

9.8.1 The EIS is used as the surrogate. The recovery limits are 20-200%.

9.9 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.

9.10 Lower Level of Quantitation:

9.10.1 LLOQs are analyzed annually.

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

9.11 Method Detection Limits

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

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

9.12 Initial Demonstration of Capability (IDC)

9.12.1 See SOP: 770032 Personnel Training.

9.12.2 IDCs are performed when:

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

9.12.2.2 There is a major change in hardware.

9.12.2.3 There is a major change in sample preparation.

9.12.2.4 There is a major change to the instrument method.

9.12.2.5 New analytes are added to the method.

9.12.3 Blind Sample IDC

9.12.3.1 Performed annually.

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

9.12.3.3 The primary chemist will analyze this spike sample.

9.12.3.4 The blind sample measured concentration should be within LCS control limits.

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

9.14 Document the preparation of samples in Element and the preparation log book.

9.15 Document all instrument problems in the instrument log book.

9.16 Print and store the sequence in the instrument log book.

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

11.2 40 CFR Part 136, Appendix B, "Definition and Procedure for the Determination of Method Detection Limit", Revision 2, 8/28/17

11.3 40 CFR Part 136.6: Method modifications and analytical requirements.

11.4 40 CFR Part 136.7: Quality assurance and quality control.

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

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

11.7 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.

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

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

11.10 Chemical Hygiene Plan, US EPA Region 10 Laboratory.

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

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

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

11.14 MEL SOP 730127: Proper Manual Peak Integration

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

11.16 MEL SOP 770032 SOP for Personnel Training

Appendix A: Compound List and Transitions

Table A01

Analyte	CAS	Quantitation Transition	Qualifier Transition	Ion Polarity
6PPD-quinone	2754428-18-5	299.1 → 215.1	299.1 -> 241.1 299.1 -> 187.1	Positive
D5-6PPD-quinone (EIS/Surrogate)	NULL	304.1 → 220.1	304.1 -> 246.1	Positive
13C6-6PPD-Quinone (IIS)	NULL	305.1 → 221.1	305.1 -> 247.1	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	7.34	D5-6PPD-quinone (EIS)
D5-6PPD-quinone (EIS/Surrogate)	7.33	13C6-6PPD-Quinone (IIS)
13C6-6PPD-Quinone (IIS)	7.34	NA

Note 1: Retention Times are approximate and can change depending on instrument conditions.
Note 2: The role of the D5-6PPD-quinone and 13C6-6PPD-Quinone may be switched. See section 5.3.1.

Appendix C: Instrument Method

Method Name: 6PPDQ_2022A.m

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

MS QQQ Mass Spectrometer Model G6460A Settings:

Table C01: 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 C02: 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 C03: 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 C04: MS Scan Parameters

Data Stg	Threshold
Centroid	0

Table C05: 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 C06: MS Chromatograms

Chrom Type	Label	Offset	Y-Range
TIC	TIC	0	1500000

Sampler Model G1329B:

Table C07: 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 C08: 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 C09: 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 C10: 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 C11: 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 D: Routine Maintenance

Routine Maintenance Schedule:

Daily Maintenance:
1. Change the needle wash solvents.
2. Replace IIS vial.
3. Check solvent eluent levels.
4. 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.
2. Run a check tune.

Monitor:
1. Rough Vac number: (1.8-2.2 torr is normal)
2. Slope. (1-3 is normal)
3. High Vac number (2.7-3.3X 10^{-5} torr is normal)
4. Collision cell gas on (3.0-6.0X 10^{-6} torr is normal)
5. Collision cell gas off

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 updates.