Standard Operating Procedure

6PPD-Quinone Soil Extraction and Analysis by Triple Quadrupole LC-MS/MS

City of Tacoma
Environmental Services Laboratory

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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 City of Tacoma.

Although the lab follows the SOP in most instances, there may be instances in which the lab uses an alternative methodology or procedure with quality assurance and management approval. Deviation will include documentation through the use of non-conforming work documents.

SOP Revision History

Revision Date	Rev Number	Summary of Changes	Sections	Reviser(s)
9/24/2024	1	New SOP	All	Fatima Alsinai, Tom Chontofalsky, Monica Herbert, Terri Torres
11/19/2024	2	Updates to extraction procedures	2.1 4.3.5, 4.3.6 5.6, 5.7 10.2.5	Fatima Alsinai, Tom Chontofalsky, Monica Herbert, Terri Torres
2/3/2025	3	Updates to extraction procedures. Added requirements for NIS/IIS Updated EIS/SS and NIS/IIS compounds to match EPA draft method 1634	6.4 and 10 8.9.2 Throughout	Fatima Alsinai, Tom Chontofalsky, Terri Torres

Related Documents

SOPs, Methods, or Manuals that support this procedure
1008_Standard and Reagent Preparation and Documentation
EPA Functional Guidelines
Variance Memo
Chemical Hygiene Health and Safety Plan
SOP 1005 Corrective Action
SOP 1017 Control Charts
EPA Draft Method 1634, Determination of 6-PPD-Quinone in Aqueous Matrices Using Liquid Chromatography with Tandem Mass Spectrometry (LC/MS/MS)
1022_Cryomill Sample Processing
EPA Definition and Procedure for Determination of the Method Detection Limit, Revision 2

Related Records

logs, labels, bench sheets, or report templates associated with this procedure
SDS Repository
Element\Print\bch_COT_OrgPrep_v1.rpt
Element\Laboratory\Standards
Element\Print\lex_COT_default.rpt
Element\Print\cub_default

1.0 Scope and Application

1.1 This SOP covers the extraction and analysis of 6PPD-Quinone (6PPD-Q) in solid matrices. 6PPD-Q is formed in the environment from the conversion of the tire additive N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (PPD) in the presence of ozone.

1.2 The method calibrates and quantifies 6PPD-Quinone using retention time, a precursor ion, a product quantifier ion, at least 1 product qualifier ion, and the ratio between these two product ions.

1.3 The analysis portion of this method is to be used by, or under the direct supervision of analysts experienced in the use of Agilent chromatography (LC-MS/MS) systems, and Mass Hunter software.

2.0 Summary of Method

2.1 Solid samples are prepared and extracted using 0.25 g of sample spiked with isotopically labeled (EIS) $13C_6$-6PPD-Quinone. Samples are extracted using sonication with Hexane and Ethyl acetate, evaporation to dryness and re-constituted in acetonitrile.

2.2 The extract is spiked with the non-extracted internal standard (NIS or IIS) D5-6PPD-Quinone solution and injected on the liquid chromatograph (HPLC) equipped with a C18 column interfaced to a tandem mass spectrometer (LC/MS/MS) in the multiple reaction monitoring (MRM) mode. The 6PPD-Q is identified by comparing the acquisition of the mass transitions and retention time to reference spectra and retention time for the calibration standards acquired under identical LC/MS/MS conditions.

2.2.1 The target analyte and the isotopically labeled standards are identified through peak analysis of the Multiple Reaction Monitoring (MRM) transitions and retention time comparison.

2.2.2 Quantitative determination of 6PPD-Q concentration is made using the primary quantitation ion (Q1) with respect to the EIS, and the results for 6PPD-Q are recovery corrected by the isotope dilution method.

2.2.3 The EIS recoveries are determined similarly against the NIS/IIS and are used as general indicators of overall analytical quality.

2.2.4 The quality of the analysis is assured through reproducible calibration and testing of the extraction and LC/MS/MS systems.

3.0 Interferences

3.1 Method interferences may be caused by contaminants in solvents, reagents, glassware and other sample processing apparatus that may yield 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 using the same conditions as for samples.

3.2 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.

3.3 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.

3.4 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.

4.0 Safety

4.1 Refer to City of Tacoma, Environmental Services Laboratory's "Chemical Hygiene Health and Safety Plan", current revision, for standard lab safety practices.

4.2 Appropriate Personal Protective Equipment (PPE) must be worn at all times. When in the laboratory, safety goggles must be worn at a minimum. When handling chemicals, reagents, or samples, a laboratory coat and gloves must be worn. Oven-safe gloves are located near the glassware oven and must be worn when handling hot glassware.

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

4.3.1 Pure standards should be handled by trained personnel, with suitable protection to skin and eyes, and care should be taken not to breathe the vapors or ingest the materials.

4.3.2 Solutions must be prepared in a hood, following universal safety measures. Make certain the vacuum exhaust hose used during the filtering is securely anchored inside of a fume hood to prevent vapors from being released into the working environment.

4.3.3 Acetonitrile – Flammable, Poison, Irritant.

4.3.4 Formic Acid – Flammable, Corrosive, Toxic, Irritant.

4.3.5 Hexane – Flammable, Toxic, Irritant

4.3.6 Ethyl acetate – Flammable, Toxic, Irritant

4.4 Read all applicable Safety Data Sheets (SDS) before using this procedure. SDS are found in paper format in the red binders across from room 220, or in electronic format at \\fspwes01\GROUP\Lab ES\Documentation\MSDS\SDSList.accdb

5.0 Equipment and Supplies

5.1 Analytical balance capable of accurately weighing to the nearest 0.1 mg.

5.2 8 mL amber screw top vials with Teflon™ lined screw tops.

5.3 Syringes and Pipettes – assorted sizes for the preparation of standards.

5.4 Nitrogen evaporation device - The N-Evap by Organomation Associates, Inc.

5.4.1 The N-Evap water bath does not need to be used, as all evaporation is done at room temperature.

5.5 4 oz Amber glass jar

5.6 12 mL amber screw top vials with Teflon™ lined screw tops.

5.7 2 mL amber screw top vials with Teflon™ lined screw tops.

5.8 Vortex mixer

5.9 Centrifuge

5.10 Ultrasonic cleaner

5.11 Liquid chromatography, triple quadrupole mass spectrometer system (LC-MS/MS). Thermo Scientific Quantiva, Agilent 6470, or equivalent.

5.12 Analytical column – A reverse phase column: Agilent InfinityLab Poroshell 120 EC-C18, 2.1 x 50 mm, 1.9 um (p/n 699675-902)

5.13 Delay Column – Agilent InfinityLab PFC Delay Column 4.6 x 30mm (p/n 5062-8100)

5.14 Agilent Masshunter data acquisition and processing system - capable of controlling the LC-MS/MS and the continuous acquisition of all mass spectra and ions obtained throughout the duration of the chromatographic program.

6.0 Reagents and Standards

Note: Reagent and Standard preparations are recorded in Element® following SOP 1008 Standard and Reagent Preparation and Documentation.

6.1 Acetonitrile – HPLC grade or equivalent.

6.2 Hexane – HPLC grade or equivalent

6.3 Ethyl acetate - HPLC grade or equivalent

6.4 Calcium Bentonite Clay - Pharmaceutical Grade

6.5 Ammonium Acetate – Ultra LCMS grade or equivalent.

6.6 Formic acid - (greater than 96% purity or equivalent), verified by lot number before use, store at room temperature.

6.6.1 Aqueous reagent A: Acetonitrile with 1mL Formic Acid (1L final volume).

6.6.2 Aqueous reagent B: DI Water, 1mL Formic Acid with Ammonium Acetate - Add 0.154g Ammonium Acetate and 1mL of Formic Acid to DI water for a final volume of 1L, resulting in a 2 mM solution. Store at room temperature, shelf life 2 months.

6.7 Mass labeled injection internal standard (NIS/IIS) D5-6PPD-Quinone (100 ug/mL): HPC Standards 688151 or equivalent. Store according to vendor specifications.

6.7.1 NIS/IIS Intermediate (2000 ng/mL): Dilute 20 uL NIS/IIS Stock to 1 mL with Acetonitrile.

6.7.2 NIS/IIS Spike (20 ng/mL): Dilute 100 uL of NIS/IIS Intermediate to 1 mL with Acetonitrile.

6.8 Mass labeled injection extractable internal standard solution / surrogate (EIS/SS) 13C6-6PPD-Quinone (100 ug/mL): Cambridge Isotope laboratories, Inc. CLM-

12293-1.2 or equivalent. Store according to vendor specifications.

6.8.1 EIS/SS Intermediate (2000 ng/mL): Dilute 20 uL of EIS/SS Stock to 1 mL with Acetonitrile.

6.8.2 EIS/SS Spike (200 ng/mL): Dilute 100 uL of EIS/SS Intermediate to 1 mL with Acetonitrile. 10 uL of NIS/IIS Spike is added to soil samples prior to extraction.

6.9 6PPD-Quinone (100 ug/mL 6PPD-Quinone): Certified standard solutions from certified standard vendors (Cambridge Isotope Laboratories ULM-12288-1.2, or equivalent). Store according to vendor specifications.

6.9.1 6PPD-Quinone Intermediate Stock (1000 ng/mL): Prepare standard by diluting 20 µL of the stock standard solution to 2 mL with Acetonitrile.

6.9.2 6PPD-Quinone ICAL Standard (5 ng/mL): Prepare standard by diluting 5 µL of the Intermediate Stock solution (6.9.1) to 1 mL with Acetonitrile.

6.9.3 6PPD-Quinone ICAL Standard (1 ng/mL): Prepare the standard by diluting 200 uL of the 5 ng/mL Intermediate Standard (6.9.2) to 1 mL with Acetonitrile.

6.9.4 ICAL Standards: Dilute the 6PPD-Quinone ICAL Std or Intermediate Stock in acetonitrile. Add 10 uL of EIS/SS Spike (6.8.2) to the calibration concentrations, resulting in a final concentration of 2 ng/mL. The suggested ICAL concentrations are 0.01, 0.025, 0.1, 0.5, 1, 2, 5, 10, 25, 50, and 100 ng/mL. All standards are also spiked with 10 µL of the NIS/IIS (6.7.2). Any standard, surrogate, or spike concentrations may differ from those stated in this SOP.

Calibration Level	uL of 1 ng/mL ICAL Std (6.9.3)	µL of 5 ng/mL ICAL Std (6.9.2)	uL of Intermediate Std (6.9.1)	µL of EIS/SS Spike (6.8.2)	uL of Acetonitrile
CAL1 (0.01 ppb iCal)*	10			10	980
CAL2 (0.025 ppb iCal)*	25			10	965
CAL3 (0.1 ppb iCal)	100			10	890
CAL4 (0.5 ppb iCal)		100		10	890
CAL5 (1 ppb iCal)		200		10	790
CAL6 (2 ppb iCal)**		400		10	590
CAL7 (5 ppb iCal)			5	10	985
CAL8 (10 ppb iCal)			10	10	980
CAL9 (25 ppb iCal)			25	10	965
CAL10 (50 ppb iCal)			50	10	940
CAL11 (100 ppb iCal)			100	10	890

*Concentration used for LCV

** Concentration used for CCV

6.10 Laboratory Control Sample (LCS): To prepare the LCS at a concentration of 25 ppb, weigh 1 g of clay (section 6.4) into an 8 mL amber glass vial and spike with 25 uL of the 1000ppb 6PPD_Quinone Intermediate Stock (6.9.1). Add 2 mL of Ethyl acetate and vortex for 10-15 secs. Completely dry the LCS by placing the vial under nitrogen flow using the N-evap at a rate of 4 LPM at room temperature (do not submerge in the water bath). This process takes approximately 1 hour. Assign an expiration date matching the stock standard.

6.11 Method Detection Limit Check Sample (MRL): Prepare MRL check with a concentration of 5 ppb: weigh 1 g of clay (section 6.4) into an 8 mL amber glass vial and spike with 5 uL of the 1000ppb 6PPD_Quinone Intermediate Stock (6.9.1). Add 2 mL of Ethyl acetate and vortex for 10-15 secs. Completely dry the MRL by placing the vial under nitrogen flow using the N-evap at a rate of 4 LPM at room temperature (do not submerge in the water bath). This process takes approximately 1 hour. The MRL Check standard should not be used past the expiration date listed on the stock standard.

6.12 6PPD-Quinone second calibration verification stock (SCV **100 ug/mL**): Certified standard stock solution HPC Standards 688152, or equivalent). Store according to vendor specifications. Note this solution is from a second-source than the calibration standard.

6.12.1 6PPD-Quinone Intermediate Verification Stock (1000 ng/mL): Prepare the standard by diluting 20 µL of the stock standard solution (6.12) to 2 mL with Acetonitrile.

6.12.2 6PPD-Quinone ICAL Verification Standard (2 ng/mL): Prepare the standard by diluting 2 µL of the Intermediate Verification Stock solution (6.12.1) and 10 uL of EIS/SS (6.8.2) to 1 mL with Acetonitrile.

6.13 When not being used, store standard solutions in the dark at less than 6°C, but not frozen, unless the vendor recommends otherwise, in screw-capped vials with PTFE-lined caps. The laboratory must maintain records of the certificates for all standards, as well as records for the preparation of intermediate and working standards, for traceability purposes. Scan these as PDFs named with the Standard ID_01 into the fs005\element\PDF\Standards\ folder.

6.14 Allow all solutions to warm to room temperature prior to use. Mix using a vortex mixer prior to taking aliquots for use. Standards should not be used past the expiration date listed on the standard.

7.0 Sample Collection, Preservation, and Handling

7.1 Collect samples in 4 oz (or larger) pre-cleaned, amber glass jars with Teflon lined lids.

7.2 All samples are protected from light, iced, or refrigerated at ≤ 6°C from the time of collection until receipt by the laboratory. Once received, the samples may be stored protected from light, at < 6 ºC until sample preparation. After extraction, sample extracts should be stored protected from light, in the refrigerator at 0 – 6 ºC while not being analyzed.

7.3 Samples must be extracted within 14 days from sample collection or 1 year, if frozen -20°C.

7.4 Extracts must be analyzed within 28 days from extraction.

8.0 Quality Control and Method Performance

8.1 Initial Demonstration of Capability (DOC) is performed by each analyst once prior to reporting sample results. The DOC is repeated if a major change is made to the extraction, analysis method or equipment or for any modifications to the method. The DOC consists of the analysis of four replicates of the Laboratory Control Sample. A least one method blank must also be included. All sample processing steps that are used for processing samples, including preparation, extraction, and

concentration, must be included in this test. Follow SOP 1017_Control Charts to generate the DOC recovery chart with average recovery and standard deviation. The DOC is acceptable if the relative standard deviation (RSD) is less than 20% and the average recoveries are within 70-130%. If any analyte fails to meet these requirements, the test may be repeated only for those analytes that failed. DOC data are stored in the analyst's training folder as a PDF file in Training Records.

8.2 Method Detection Limit (MDL) is defined 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. It is determined the first time the method is performed on each instrument and repeated annually, or if there is a major change in the procedure or equipment. For new instrumentation, analyze a minimum of two spiked replicates and two method blank replicates on the new instrument. If both method blank results are below the existing MDL, then the existing MDL is validated. Combine the new spiked sample results to the existing spiked sample results and recalculate the MDLs. If the recalculated MDLs is within 0.5 - 2.0 times the existing MDL, then the existing MDLs is validated. For ongoing annual verification, perform at least 7 replicates, two per quarter, per instrument is suggested. Enter these samples as MRL Check Samples in the preparation bench sheet. The MDLs is recalculated every 13 months using the previous 2 years of MDLs data as:

$$\mathrm{MDL_s} = \mathrm{t}_{(n-1,\ 1-\alpha=.99)} S_s$$

where:

$t_{(n-1,\ 1-\alpha = 0.99)}$ = the Student's *t*-value appropriate for the single-tailed 99th percentile *t* statistic and a standard deviation estimate with n-1 degrees of freedom.

S_s = sample standard deviation of the replicate spiked sample analyses

Additionally, the MDL_b is determined every 13 months using Method Blank data for the prior two years to include all BLKs associated with the analysis (but not those associated with rejected or re-analyzed client samples); the laboratory has the option to use only the last 6 months of method blank data, or the 50 most recent method blanks, whichever is greater. If none of the method blanks give numerical results for an individual analyte, the MDL_b does not apply. If some (but not all) of the method blanks for an individual analyte give numerical results, set the MDL_b equal to the highest method blank result. If more than 100 method blanks are available, set MDL_b to the level that is no less than the 99th percentile of the method blank results. If all of the method blanks for an individual analyte give numerical results, then calculate the MDL_b as:

$$\mathrm{MDL_b} = \overline{\mathrm{X}} + \mathrm{t}_{(n-1,\ 1-\alpha=.99)} S_b$$

where:

$\overline{X}$= mean of the method blank results (use zero if the mean is negative)

$t_{(n-1,\ 1-\alpha = 0.99)}$ = the Student's *t*-value appropriate for the single-tailed 99th percentile *t* statistic and a standard deviation estimate with n-1 degrees of freedom.

S_b = sample standard deviation of the replicate method blank sample analyses

The verified MDL is the greater of the MDLs or MDLb. If the verified MDL is within 0.5 to 2.0 times the existing MDL, and fewer than 3% of the method blank results (for the individual analyte) have numerical results above the existing MDL, then

the existing MDL may be left unchanged. Otherwise, adjust the MDL to the new verification MDL. (The range of 0.5 to 2.0 approximates the 95th percentile confidence interval for the initial MDL determination with six degrees of freedom.)

8.3 Create a MRL standard (section 6.11). Extract and analyze the MRL Check in the same manner as samples. MDL results are imported to Element/DataTool for the MDL calculation. Recovery of the MRL must be within 70-130%. MDL data is stored on \\fspwes01\Transfer\Organic MDLs as a PDF file. For additional details, refer to Definition and Procedure for Determination of the Method Detection Limit, Revision 2 and the laboratory SOP 1016 Determining and Applying Method Detection Limits.

8.4 Blind to the Analyst (BTTA) Study is performed annually, at a minimum.

8.4.1 A 1 g sample made using clay (section 6.4) that is spiked by a chemist who will not perform the extraction or analysis at a concentration known only by that chemist and the QA Manager.

8.4.1.1 Measure 1 g clay into an 8 mL amber glass vial. Spike the sample with a known quantity of standard using gas tight syringe. Add 2 mL of Ethyl acetate and vortex for 10-15 secs. Completely dry the sample by placing vial under nitrogen flow (4 LPM) using N-evap at room temperature (do not submerge in the water bath).

8.4.1.2 The chemist must document how the sample was made, including volumes, concentrations, standards, IDs, date made, sample concentration and who made the sample.

8.4.2 The sample will be logged in to LIMS for 6PPD-Q analysis, labeled and placed in the cooler, as with any other sample. The sample is to be extracted and analyzed like any other client sample.

8.4.3 Follow the normal review process for the BTTA sample, as would be performed for any other sample, including variance memos for any QC failures or anomalies.

8.4.4 Following extraction and analysis, the QA manager will review the results and grade the sample reports. The graded report is submitted by the QA manager to Washington State Department of Ecology.

8.5 Instrumental Blanks (990uL acetonitrile, 10uL EIS and 10uL NIS only): Prior to the start of the analytical sequence, analyze an instrument blank (IBL) (acetonitrile spiked with EIS & NIS) to ensure no instrument contamination has occurred. In addition, an instrument blank should be analyzed any time carryover contamination is suspected (i.e., after a sample with high analyte concentrations is analyzed), and when new lots of solvent are purchased.

8.6 Method Blank (Batch#-BLK#): Analyze at least one method blank (BLK) per sample batch or at least 5% of samples prepared – equivalent to 1 per 20 field samples or less. The BLK is made up using Clay (section 6.4) and extracted using the same preparation procedure as the associated samples. The blanks must be free from contamination at a concentration at 1/2 the reporting limit (RL/MRL). If contamination is shown in the Method Blank, halt sample analysis and correct the issue. Samples affected by the Method Blank should be re-analyzed, or qualified if re-analysis is not possible.

8.6.1 If the blank contains a concentration greater than the MDL and the sample

concentration is less than the reporting limit, report the MRL value with a "U".

8.6.2 If the sample concentration is greater than 10 times the blank value, no data qualification is required.

8.6.3 If the sample concentration is greater than or equal to 5 times the blank value but less than 10 times the blank value, qualify the data as estimated with a “J”.

8.6.4 If the sample concentration is less than or equal to 5 times the blank value, qualify the data as not detected at or above the associated estimated concentration with a “UJ”.

8.6.5 If gross contamination exists in the blank, positive sample results may require rejection and be qualified as unusable, "R". Non-detected target compounds do not require qualification unless the contamination is so high that it interferes with the analyses of non-detected compounds. Unusable data may require re-extraction.

8.6.6 Complete a QC Variance form for any result requiring qualification.

8.7 Laboratory Control Sample (LCS) or Standard Reference Material (Batch#-BS#): A laboratory control sample (LCS) is created (section 6.10) and extracted and analyzed at a frequency of one per batch or every 20 samples. The acceptable recoveries are 70-130%. Re-analyze samples associated with any LCS failures or qualify the data where reanalysis is not possible. After the analysis of 30 LCS samples, the lab limits are updated on a regular basis based on 2 standard deviations from the mean.

8.7.1 Reanalyze LCS if recovery is outside the criteria after evaluating whether system maintenance could improve recovery and taking any actions indicated. Analyze a second LCS if one was extracted with the batch. Evaluate repeat analyses only for the analytes that failed the initial analysis. Consult with Environmental Laboratory Scientist (ELS) III if recovery is still outside the criteria to determine whether re-extraction is possible within sample holding times. Report data associated with the best recovery.

8.7.2 Qualify results with as estimated “J” for detects and unusable “R” for non-detects, if the LCS recovery is less than the lower recovery limit. Complete a QC Variance form.

8.7.3 Results qualified as unusable “R” are not usable for regulatory purposes. Notify the Project Client for further action.

8.7.4 Qualify results as estimated “J” for detects and complete a QC Variance form if the LCS recovery exceeds the upper recovery limit. Do not qualify non-detects.

8.7.5 Complete a QC Variance form for any result requiring qualification.

8.8 Matrix Spike/Matrix Spike Duplicate (Batch#-MS# and Batch#-MSD#): ***A matrix spike is analyzed only if requested by the client.*** The spike recoveries should be within laboratory control limits of 50-150% with an RPD of 50%.

8.8.1 Do not qualify results if the sample concentration exceeds the spike concentration by a factor of four or more. Do not report the MS/MSD if the parent sample concentration exceeds the upper calibration limit.

8.8.2 Reanalyze the MS or MSD if recovery is outside the criteria after evaluating whether LC/MS-MS system maintenance could improve recovery and taking any actions indicated. Consult with the senior analyst if recovery is still outside the criteria to determine whether re-extraction is possible within sample holding times. Report data associated with the best recovery.

8.8.3 If the MS and MSD recoveries are less than the lower recovery limit or ≤10%, whichever is lower, qualify results of the source sample as estimated “J” for detects and unusable “R” for non-detects. Qualify non-detects as “UJ” if recoveries are less than the lower recovery limit but not less than 10%. Complete a QC Variance form.

8.8.4 If the MS and MSD recoveries exceed the upper recovery limit, qualify results of the parent MS/MSD sample as estimated “J” for detects and complete a QC Variance form. Do not qualify non-detects.

8.8.5 If the RPD exceeds the limit, qualify detected analyte results of the source MS/MSD sample as estimated “J”, and complete a QC Variance form. Do not qualify non-detects.

8.8.6 Complete a QC Variance form for any result outside control limits or any MS/MSD not reported due to concentration of parent sample.

8.9 Sample duplicate (Batch#-DUP#). ***A duplicate is analyzed only if requested by the client.*** The duplicate relative percent difference (RPD) should be less than or equal to 50% for sample duplicates and matrix spike duplicates. If the RPD fails due to inhomogeneity or matrix interference, qualify the failing analytes in the source sample.

8.10 Extracted Internal Standard/Surrogate (EIS/SS): To assess method performance on the sample matrix, the laboratory must spike all samples with the isotopically labeled compound standard solution (EIS/SS) and all sample extracts with the non-extracted Internal Standards (NIS/IIS) spiking solution. The recovery limits for the EIS/SS are 25-200%. After the analysis of at least 30 samples, the limits are updated on a regular basis based on 2 standard deviations from the mean.

8.10.1 Analyze each sample according to the procedures in Section 10.0. Peak responses of the quantitation and confirmation ions must be at least three times the background noise level (S/N 3:1). The quantitation ion must have a S/N ≥ 10:1 if there is no confirmation ion. Retention times must fall within ± 0.4 minutes of the predicted retention times from the midpoint standard of the ICAL or initial CCV, whichever was used to establish the RT window position for the analytical batch. Compute the percent recovery of the isotopically labeled compound using the non-extracted internal standard method. The recovery of must be within 25-200% or current limits.

8.10.2 If the recovery of the EIS/SS falls outside of these limits, method performance is unacceptable for 6PPD-Q in that sample. Additional cleanup procedures must then be employed to attempt to bring the recovery within the normal range. If the recovery cannot be brought within

the normal range, the extracts may be diluted.

8.10.2.1 The sample may only be diluted to the level that the EIS/SS meet the S/N and retention time requirements and are still recovered at greater than 5%. For example, if the EIS/SS recovery of the affected analyte in the undiluted analysis is 50%, then the sample cannot be diluted more than 10:1; if the EIS/SS recovery of the affected analyte in the undiluted analysis is 30%, then the sample cannot be diluted more than 6:1. If sample extract dilution does not correct the recovery, a new extraction should be performed.

8.10.3 If after dilution and/or re-extraction any EIS/SS recovery is outside limits and there is no further sample available for extraction or dilution, qualify the 6PPD-Q in the analysis as indicated below. Use professional judgment if sample dilution is a factor in EIS/SS recovery.

8.10.3.1 For target analyte detections where the recovery exceeds the upper recovery limit qualify results as estimated, “J". Do not qualify non-detects.

8.10.3.2 If the recovery is less than the lower recovery limit but greater than 10%, qualify results as estimated “J".

8.10.3.3 If the recovery is less than 10% or the lower limit in Table 8 whichever is less, qualify results as “J" for detects and unusable “R” for non-detects.

8.11 Non-Extracted Internal Standards (NIS/IIS): Retention times must fall within ± 0.4 minutes of the predicted retention times from the midpoint standard of the ICAL or opening CCV, whichever was used to establish the RT window position for the analytical batch. The NIS/IIS area in the field samples and QC samples must be within 50-200% of the most recent CCV, the most recent CCV must be within 50-200% of the mean area of the calibration.

8.11.1 If sample dilution is required, the NIS/IIS response in the diluted extract is no longer required to be within ±50% of the response (peak area) in the most recent CCV.

8.12 Control Charts – Control charts are maintained in Element® according to SOP 1017_Control Charts.

8.13 Corrective Action – QC results outside of limits and deviations from the SOP are documented according to SOP 1005_Corrective Action.

8.14 See a QC guidance summary table in Appendix A.

9.0 Calibration and Standardization

9.1 Refer to the following SOPs for calibration of balances and thermometers, instrument, glassware cleaning procedures, and the preparation of reagents and standards.

1006_Reagent Preparation and Documentation

1008_Standards and Reagent Preparation and Documentation

1011_Glassware Cleaning

1013_Thermometer Calibration and Temperature Control

1015_Analytical Balance Calibration and Maintenance

9.2 Calibration and Standardization

9.2.1 Calibration is required whenever the laboratory takes an action that changes the chromatographic conditions or might change or affect the initial calibration criteria, or if either the CCV or Instrument Sensitivity Check (LCV) acceptance criteria have not been met.

9.2.2 The instrument must have a valid mass calibration following the manufacturer specified procedure prior to initial sample analysis and at least annually following this initial calibration. The mass calibration is updated on an as-needed basis (QC failures, ion masses fall outside of the instrument required mass window or major instrument maintenance). Record maintenance in Element® Instrument Maintenance Log under the instruments name.

9.2.3 Mass calibration must be verified after each mass calibration, prior to any sample analysis. Mass calibration must be performed per the instrument manufacturer's instructions. A mass calibration verification must be performed using standards whose mass range brackets the masses of interest (quantitative and qualitative ions). Record maintenance in Element® Instrument Maintenance Log under the instruments name.

9.2.4 Instrument Tune

9.2.4.1 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.

9.2.4.2 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 fails, then instrument maintenance and/or a full autotune are required.

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

9.2.5 Perform initial calibration and repeat if any major changes or maintenance (column removal or replacement, etc.) are performed or if continuing calibration fails. Record maintenance in Element® Instrument Maintenance Log under the instruments name.

9.2.5.1 Prepare calibration standards at a minimum of seven concentration levels with the lowest standard at or below the MRL (this point can be used as the instrument sensitivity check (see section 9.7). The initial calibration solutions contain the entire suite of EIS/SS and NIS/IIS compounds, and target analytes. All solvents used in standard preparation need to be LCMS grade or better.

9.2.5.2 Sufficient instrument sensitivity is established if a signal-to-noise ratio ≥ 3:1 for the quantification ions and the confirmation ions for the target analyte, or ≥ 10:1 for the EIS/SS and NIS/IIS, which only

have a quantification ion, can be achieved when analyzing the lowest concentration standard within the quantitation range that the laboratory includes in its assessment of calibration linearity.

9.2.5.3 Calculations are performed by the instrument's software. Agilent MassHunter Software has many options for calibration curves which may be used. The calibration must meet or exceed one of the following two criteria. *Note that when using a weighted regression or non-linear regression calibration, the regression must be weighted inversely proportional to concentration and must not be forced through zero. Additionally, when using a weighted regression calibration, linearity must be assessed using Option 2*:

Option 1: Calculate the relative standard deviation (RSD) of the RF values of the initial calibration standards. The RSD must be ≤ 20% to establish instrument linearity.

$$mean\ RR\ or\ RF = \frac{\sum_{i=1}^{n}(RR\ or\ RF)i}{n}$$

$$SD = \sqrt{\frac{\sum_{i=1}^{n}(RR\ or\ RFi - mean\ RR\ or\ RF)^2}{\mathrm{n}}}$$

$$RSD - \frac{SD}{mean}x100$$

where:
RR or RFi = RR or RF for calibration standard i
n = Number of calibration standards

Option 2: Calculate the relative standard error (RSE) of the initial calibration standards. The RSE for all method analytes must be ≤ 20% to establish instrument linearity.

$$RSE = 100\ x \sqrt{\sum_{i=1}^{n} \frac{\left[\frac{x'_i - x_i}{x_i}\right]^2}{n-p}}$$

where,
x_i = Nominal concentration (true value) of each calibration standard
x'_i = Measured concentration of each calibration standard
n = Number of standard levels in the curve
p = Type of curve (2 = linear, 3 = quadratic)

1.1.1 Response factor (RF) for the Extracted Internal Standard (EIS/SS) compound:

$$RF_s = \frac{A_{EIS}}{A_{NIS}} \times \frac{C_{NIS}}{C_{EIS}}$$

where: A_{EIS} = Area of EIS/SS

C_{EIS} = Concentration of EIS/SS

A_{NIS} = Area of Non-Extracted Internal Standard (NIS/IIS)

C_{NIS}= Concentration of NIS/IIS

9.3 Non-extracted Internal Standard Area

9.3.1 Each time an initial calibration is performed, use the data from all the initial calibration standards used to meet the linearity test in Section 9.2. Record the area for the NIS/IIS compound of the mid-point of the calibration for use in evaluating opening CCV results for sample analyses.

9.4 Retention Time window position and width (Once per ICAL and at the beginning of the analytical sequence).

9.4.1 Position of method analyte, EIS/SS analyte, and NIS/IIS analyte peaks shall be set using the midpoint standard of the ICAL curve when ICAL is performed. On days when ICAL is not performed, the initial CCV retention times or the midpoint standard of the ICAL curve can be used to establish the RT window position.

9.4.2 Method analyte, EIS/SS analyte, and NIS/IIS analyte RTs 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.

9.5 Independent Calibration Verification (ICV/SCV). 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, preferably an alternate vendor is used (section 6.12). If an alternate vendor is not available, a different lot number from the same vendor may be used.

9.5.1 Analyze the ICV standard directly after calibration. The ICV is evaluated with every new calibration curve that is analyzed.

9.5.2 The analyte recovery should be within +/- 30% of the 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.

9.6 Back Calculation (Residuals). Re-calculate each standard concentration level using the updated, and passing, 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 10% of analytes are allowed to be within 50%. If these requirements are not met, the ICAL for those analytes fails and should be reanalyzed.

9.6.1 If the initial calibration does not meet the acceptance criteria, any sample results associated with the out-of-control analyte(s) should be qualified as estimated “J.”

9.7 Instrument Sensitivity Check / Low-Level Continuing Calibration Verification (LCV).

9.7.1 Analyze a Low-Level continuing calibration verification (LCV) standard at a minimum prior to the analysis of samples and blanks. The LCV analyzed is a MRL-level standard and should be prepared from the same source as calibration standards. The acceptance range is +/- 50%, signal-to-noise ≥ 3:1 for quantitation and confirmation ions and an ion ratio of ± 50%. If a LCV does not meet quality criteria, analysis must be halted, and the sensitivity of the LC/MS/MS system adjusted before analysis of field or QC samples

9.8 Continuing calibration Verification (CCV).

9.8.1 Analyze a continuing calibration verification (CCV) standard at a minimum prior to the analysis of samples and blanks, at the end of an analytical run sequence containing samples, and after every 10 field samples. The exception is after an acceptable initial calibration is run, when 10 samples may be analyzed before a CCV is required. 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 all samples following the failing CCV. If an analyte in the CCV failed because of high recovery, but that analyte was not detected in a sample extract, then that sample extract need not be reanalyzed. On a case-by-case basis, samples associated with a CCV not meeting acceptance limits can be reported as long as they are qualified as estimates. Note draft EPA method 1634 uses the term calibration verification (VER) instead of CCV.

9.8.2 Complete a QC Variance form for any result requiring qualification.

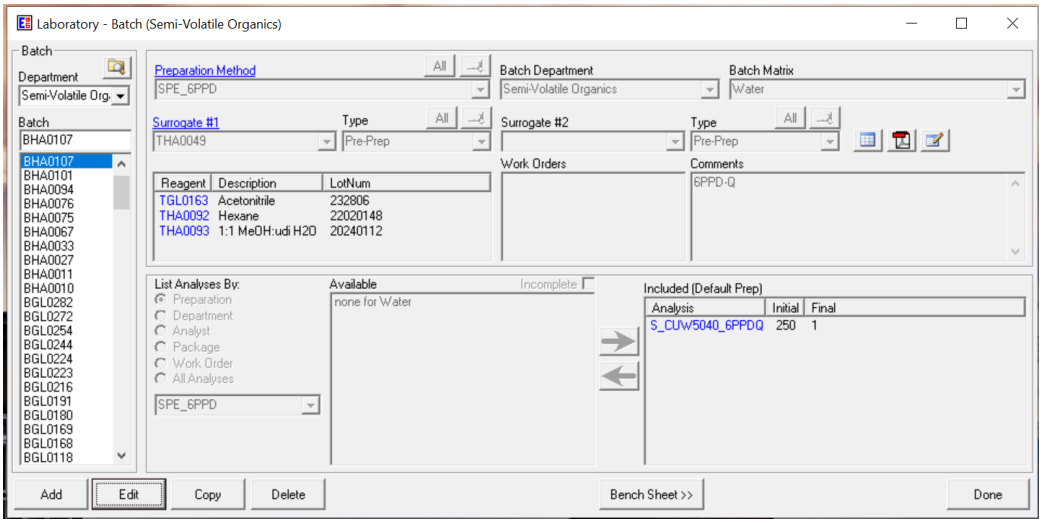
9.9 The lowest level calibration standard must meet a signal-to-noise ratio of 3:1 for the quantitation ions and confirmation ions, and 10:1 for quantitation ions that have no confirmation ion and be at a concentration less than or equal to the LOQ.

10.0 Procedure

10.1 Cleaning of glassware, tools, and surfaces. Satisfactory cleaning may be accomplished by rinsing with acetonitrile, and then washing with Alconox and water.

10.2 Review Extraction Bench Sheets prior to sample preparation. Environmental Laboratory Scientist (ELS) II or III signs and dates on the Analyst Approval line. Note that in the sample comments column on the bench sheet,

10.2.1 In Element: Laboratory > Batch > Add



Select the appropriate Preparation Method, Batch Matrix, Surrogate and Analysis. Add reagents by right clicking in the Reagent box.

Save > Bench Sheet>>

The Bench Sheet will be called up. Select Edit > Add to add client samples by container, and any applicable QC samples. Right click highlighted samples to change Initial/Final volumes, Surrogate Amounts, Spike IDs/amounts and select Source Samples for all MS/MSD and DUP (if required).

Select Save, then the print icon.

10.2.2 For sediment samples with limited sample volume follow SOP 1022_Cryomill Sample Processing prior to extraction.

10.2.3 Samples should be air dried prior to extraction.

10.2.4 Prepare samples including (method blank, LCS (BS), MRLs, and SRMs) by weighing approx. 0.2 g to 0.25 g of sample into an 8 mL amber glass vial.

10.2.5 Spike all samples with 10 µL of EIS/SS (surrogate) (section 6.8.2).

10.2.6 The samples are now ready for extraction.

10.2.6.1 Add 4 mL of Hexane and 0.5 mL of Ethyl Acetate to sample vial.

10.2.6.2 Vortex for 30 seconds.

10.2.6.3 Sonicate for 30 minutes.

10.2.6.4 Centrifuge sample for 5 minutes at 2000 rpm.

10.2.6.5 Manually decant the solvent layer into a 12 mL vial. Carefully pour it in to avoid spilling. Place the 12 mL vial under the N-evap at 4 LPM at room temperature (not in the water bath) until the Hexane/Ethyl Acetate is completely evaporated off.

10.2.6.6 Using a pipette, re-elute extract with 1mL of Acetonitrile.

10.2.6.7 Vortex for 15-20 seconds and sonicate for 2 mins.

10.2.6.8 Transfer the 1 mL extract into a 2 mL amber glass vial. To each 1 mL volume, add 10 µL of non-extracted internal standard (NIS/IIS) spiking solution, section 6.7.2. Cap the vial, shake or vortex to mix well, and then transfer to the LC/MS-MS for analysis. Refrigerate extract if not analyzed right away.

10.3 If the Mass detector system has been turned off: turn on the mass detector.

10.4 If needed, clean source prior to analysis. Be sure to cool source before opening, then rinse and wipe down interior of the spray chamber with isopropyl alcohol. Sonicate source transfer tube in a mixture of 50% DI water and 50% isopropyl alcohol. Dry the transfer tube before installing. Maintenance may vary depending on the level of cleanliness. See manufacture user guide.

10.5 See Appendix A for maintenance information.

10.6 Turn on autosampler, pump and degas unit. If the HPLC has been idle and the reservoirs are empty or new reagent has been added to the reservoir(s), prime the pumps by opening the pump bypass valve and pressing the purge button. Close valve after pump is primed. Priming procedure may vary between instruments.

10.7 Start Triple Quadrupole (Agilent MassHunter) software. Ensure that all systems are communicating, and status lights are green. Load the correct acquisition software method.

10.8 Instrument Setup. See Appendix B for a printout of the current method.

10.9 Recommended analytical run sequence is:

- Instrument Blank
- Instrument Sensitivity Check/ Low-Level CCV (LCV)
- CCV
- Method Blank
- LCS
- Up to 10 injections of sample extracts, diluted extracts, laboratory duplicate extracts and MS/MSD extracts (if requested)
- CCV
- Instrument Blank
- Up to 10 more injections of sample extracts, diluted extracts, laboratory duplicate extracts, and MS/MSD extracts (if requested)
- CCV

10.10 Qualitative Identification of Target Compounds

10.10.1 Target compound identification is made by precursor and product ions as well as retention time matching. A current laboratory-generated standard must be present and compared.

10.10.2 Using available software, search for the target compound in the established retention time window. Examine chromatograms and determine if a positive identification is present. See references for more information.

10.10.3 Examine baseline and peak integration to insure proper area integration. If the compound is present but not properly integrated, then manually

integrate the peak. Retain the original peak integration with the properly integrated peak with the sample data.

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

10.10.5 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.

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

10.10.7 Peak responses for 6PPD-Q must be at least three times the background noise level (signal-to-noise ratio [S/N] ≥3:1) and the EIS/SS response must have S/N of at least 10:1. If the S/N ratio is not met due to high background noise, the laboratory must correct the issue (e.g., perform instrument troubleshooting and any necessary maintenance , such as cleaning the ion source, or replacing the LC column) and the instrument must be recalibrated (Section 9.0). If the S/N ratio is not met, but the background is low, then the analyte is to be considered a non-detect.

10.10.8 6PPD-Q must elute within ± 0.1 minutes of the EIS/SS.

10.10.9 The relative retention times (RRTs) must be within ±0.4 RRT units of the standard RRT. Use professional judgment when there is a question if 0.4 RRT units may be too broad, or too narrow.

10.10.10 Verify the presence of product ions and check their corresponding ratios of the analyte in the sample. The acceptance window for the ion abundance ratio of each target analyte above the MRL is 50% to 150% of the mid-point calibration standard. The acceptance window for the ion abundance ratio of each target analyte below the MRL is 50% to 150% of the daily CCV standard. The relative response ratio is calculated by dividing the qualifier/confirmation ion area by the quantifier ion area.

10.11 Quantitative analysis of target analytes

1.1.2 When a compound has been identified, the quantification of that compound will be calculated using extracted internal standard (isotope dilution) calibration. Isotope dilution calibration requires the determination of relative response (RR) defined by the following equation.

$$RR = \frac{A_t(C_t)}{A_{EIS}(C_{EIS})}$$

Where:

A_t = Target Compound Area

C_t = Target Compound Concentration

A_{EIS} = Extracted Internal Standard/Surrogate Area

C_{EIS} = Extracted Internal Standard/Surrogate Concentration

10.11.1 For the analytes determined by isotope dilution, the relative response (RR) (labeled to native) vs. concentration in the calibration solutions is computed over the calibration range according to the procedures described below. A minimum of six calibration points are employed. The calculation of each analyte concentration, relative to its labeled analog, is determined using the area responses of the primary product ions for each calibration standard. This calculation is usually done by the analytical software.

10.11.2 Keep manual integration of peaks to a minimum following SOP 1026 Manual Integration.

10.11.3 If the response for any compound exceeds the highest calibration standard, dilute the extract (see section 8.0), and reanalyze. Target dilutions to fall between the mid-point and top of the calibration range. Adjust the volume of internal standard added proportionately to the volume of solvent added when diluting an extract already spiked with internal standard.

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

10.11.5 Solids

$$C_F = \frac{C_I(V_F)(D)(1000)}{w\left(\frac{S}{100}\right)}$$

Where: C_F = Final Concentration (ug/kg)

C_I = On Column Concentration (ng/mL)

V_F = Final Volume of Extract (mL)

D = Dilution Factor

w = Weight of Sample Extracted (g)

S = Percent Solids

11.0 Pollution Prevention and Waste Management

11.1 Store vials for disposal containing sample extracts or expired standards in the designated waste containers for this satellite area until disposed of by lab pack in accordance with the Dangerous Waste Management Plan. Keep in-use waste disposal container under the hood in Room 230. This is the designated satellite collection area for this waste stream. When waste container is full, notify the Hazardous Waste Manager for removal to Hazardous Waste Storage area. Refer to the waste stream fact sheets for details: CH1130589 (Organic extractions waste & expired standards in vials).

11.2 Collect waste solvents in an appropriate waste container and dispose of in accordance with the Dangerous Waste Management Plan. Refer to the waste stream fact sheet for details: CH573177 (Waste solvents).

11.3 Upon extraction, the solid sample waste is collected and disposed following the Dangerous Waste Management Plan. Disposal details are outlined in the waste

stream fact sheet: 0006 (non-hazardous solid waste).

12.0 References

12.1 US EPA Functional Guidelines: http://www.epa.gov/sites/production/files/2015-03/documents/somnfg.pdf

12.2 City of Tacoma Environmental Services Chemical Hygiene Health and Safety Plan, current revision

12.3 City of Tacoma Environmental Services Quality Assurance Manual, current revision

12.4 City of Tacoma Environmental Services Dangerous Waste Disposal Manual, current revision

13.0 Tables, Diagrams, Flowcharts and Validation Data

Appendix A: QC guidance table

Appendix B: Routine Maintenance Schedule

Appendix C: Instrument Setup

Appendix A: QC guidance table

QC Parameter	Frequency	Criteria	Corrective Action	Qualification
Mass Calibration	The mass calibration must be performed at least annually on an as-needed basis (e.g., QC failures, ion masses fall outside of the instrument required mass window, major instrument maintenance, or if the instrument is move).	Instrument must have a valid mass calibration following the manufacturer specified procedure prior to any sample analysis.	Problem must be corrected. No samples may be analyzed under a failing mass calibration.	NA
Mass Calibration Verification	Mass calibration must be verified after each mass calibration, prior to any sample analysis. Mass calibration must be performed per the instrument manufacturer's instructions.	A mass calibration verification must be performed using standards whose mass range brackets the masses of interest (quantitative and qualitative ions). Check the mass calibration by measuring the amount of peak drift from the expected masses. If the peak apex has shifted more than approximately 0.2 Da, recalibrate the mass axis following the manufacturer's instructions.	Problem must be corrected. No samples may be analyzed under a failing mass calibration verification.	NA
Retention Time (RT) window position establishment	Once per ICAL and at the beginning of the analytical sequence.	Position of method analyte, EIS/SS analyte, and NIS/IIS analyte peaks shall be set using the midpoint standard of the ICAL curve when ICAL is performed. On days when ICAL is not performed, the initial CCV retention times or the midpoint standard of the ICAL curve can be used to establish the RT window position.	Correct problem and reanalyze samples.	NA
Retention Time (RT) window width	Once per ICAL and at the beginning of the analytical sequence.	6PPD-Q must fall within ± 0.1 minutes of the NIS/IIS. 66PP-Q, NIS/IIS and EIS/SS also must fall within 0.4 minutes of the predicted retention times from the midpoint standard of the ICAL or initial daily CCV.	Correct problem and reanalyze samples.	NA

QC Parameter	Frequency	Criteria	Corrective Action	Qualification
Initial Calibration (ICAL)	At instrument set-up and after ICV or CCV failure, prior to sample analysis, at a minimum annually.	Initial calibration is performed using a series of at least six solutions, with at least five of the six calibration standards being within the quantification range, and with the lowest standard at or below the LOQ. (If a second-order calibration model is used, then one additional concentration is required, with at least six of the seven calibration standards within the quantitation range.) The initial calibration solutions contain the EIS/SS, NIS/IIS and target compound. Sufficient instrument sensitivity is established if a signal-to-noise ratio ≥ 3:1 for the quantitation ions and the confirmation ions, or ≥ 10:1 if the analyte only has a quantitation ion, can be achieved when analyzing the lowest concentration standard within the quantitation range that the laboratory includes in its assessment of calibration linearity ICAL must meet one of the two options below: Option 1: The RSD of the RFs or RRs for all method analytes, isotopically labeled compounds and EIS/SS must be ≤20%. Option 2: The% RSE for all method analytes, isotopically labeled compounds and EIS/SS must be ≤20%.	No samples shall be analyzed until ICAL has passed.	NA
Extracted Internal Standard/Surrogate (EIS/SS)	Must be added to every field sample, standard, blank, and QC sample.	Recovery of the EIS/SS is calculated by internal standard quantification against the NIS/IIS using Response Ratios or Response Factors. Recovery criteria for EIS/SS is 25-200%.	Correct problem. If required, re-extract and reanalyze associated field and QC samples. If recoveries are acceptable for QC samples, but not field samples, the field samples must be re-extracted and analyzed (greater dilution may be needed).	Apply J qualifier and discuss in the Case Narrative only if reanalysis confirms failures.

QC Parameter	Frequency	Criteria	Corrective Action	Qualification
Non-Extracted Internal Standard/Injection Internal Standards (NIS/IIS)	Must be added to every prepared field sample, standard, blank, and QC sample prior to instrumental analysis. NIS/IIS analyte recovery is calculated determined against the average NIS/IIS analyte area of the ICAL standards.	Retention times must fall within ± 0.4 minutes of the predicted retention times from the midpoint standard of the ICAL or opening CCV, whichever was used to establish the RT window position for the analytical batch. The NIS/IIS area in the field samples and QC samples must be within 50-200% of the most recent CCV, the most recent CCV must be within 50-200% of the area of the mid-point of the calibration.	Correct problem and reanalyze samples.	NA
Initial Calibration Verification (ICV):	After each Initial Calibration (ICAL), prior to sample analysis; analyze a second source standard.	Calculated concentration must be within ±30% of the true value.	No samples shall be analyzed until calibration has been verified.	NA
Continuing Calibration Verification (CCV)	Prior to sample analysis, after every 10 field samples, and at the end of the analytical sequence.	Calculated concentrations must be within ±30% of their true value.	Correct problem and reanalyze samples. If reanalysis cannot be preformed, data must be qualified and explained in a variance memo.	Apply J qualifier to all results for the specific analyte(s) in all samples since the lab acceptable CCV.
Instrument Sensitivity Check / Low-Level Continuing Calibration Verification (LCV)	Prior to ICAL or sample analysis	The signal-to-noise ratio of ≥ 3:1 for the quantification ions and the confirmation ions for the target analyte and ≥ 10:1 for the EIS/SS and NIS/IIS. Calculated concentration of 6PPD-Q must be within ±50% of true value.	Correct problem and reanalyze samples.	NA
Instrumental Blanks (acetonitrile only)	Immediately following the highest standard analyzed, following any sample with a high concentration where carryover is suspected, each new solvent lot and daily prior to sample analysis.	Concentration target analyte detected must be ≤ the LOQ.	No samples shall be analyzed until instrument blank has met acceptance criteria. Note: Successful analysis following the highest standard analyzed determines the highest concentration that carryover does not occur.	NA

QC Parameter	Frequency	Criteria	Corrective Action	Qualification
Method Blank (MB)	One per preparatory batch.	No method analyte can be detected ½ > MRL or >1/10th the amount measured in field samples in the batch, whichever is greater.	Correct problem, re-extract and reanalyze MB and all QC samples and field samples processed with the contaminated blank. If reanalysis cannot be performed, data must be qualified and explained in a variance memo.	Apply J qualifier to all results for the specific analyte(s) in all samples in the associated preparatory batch.
Laboratory Control Sample/Laboratory Control Sample Duplicate (LCS/LCSD)	One per preparatory batch.	Blank spiked with the target analyte. Recovery criteria is 70-130%.	Correct problem, re-extract and reanalyze LCS and all QC samples and field samples for failed analytes if sufficient sample material is available. If reanalysis cannot be performed, data must be qualified and explained in a variance memo.	Apply J qualifier to all results for the specific analyte(s) in all samples in the associated preparatory batch.
MRL Check Standard	One per preparatory batch.	Blank spiked with all analytes at concentrations one to two times the LOQ. Recovery criteria is 50-150%.	Correct problem, re-extract and reanalyze LCS and all QC samples and field samples for failed analytes if sufficient sample material is available. If reanalysis cannot be performed, data must be qualified and explained in a variance memo.	Apply J qualifier to all results for the specific analyte(s) in all samples in the associated preparatory batch.

Appendix B. Routine Maintenance Schedule:

Daily:

- Prime both pump channels prior to running.
- Fill Eluent bottles, needle wash bottle, seal wash bottle.
- Rinse the ionization chamber with wet (isopropyl alcohol) kim wipe. Check that Instrument Tune is not expired.
- Empty eluent collection bottle.

Check:

- Source Pressure (3.5-4.0 torr is normal)
- High Vac number (9.4×10^{-5} torr is normal)
- Collision cell gas on (1.7×10^{-5} torr is normal)

Weekly:

- Check and drain rough pump reservoir.
- Clean and replace ionization transfer capillary.
- Clean ionization chamber cones.

As Required:

- Update Software
- Run instrument tune
- Preventive Maintenance every year – performed by the instrument manufacturer.

Appendix C: Instrument Setup

Acquisition Method Report

Agilent | Trusted Answers

MS QQQ Mass Spectrometer

Ion Source	AJS ESI	**Tune File**	D:\MassHunter\Tune\QQQ\G6470B\Autotune_20250131_083102\atunes_20250131_092114.TUNE.XML
Stop Mode	No Limit/As Pump	**Stop Time (min)**	No limit
Time Filter	On	**Time Filter Width (min)**	0.05
LC->Waste Pre Row	N/A	**LC->Waste Post Row**	5

Time Segments

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

Time Segment 1

Scan Segments

Cpd Group	Cpd Name	ISTD?	Prec Ion	MS1 Res	Prod Ion	MS2 Res	Frag (V)	CE (V)	Cell Acc (V)	Ret Time (min)	Ret Window
EIS	13C6-6-PPD-Quinone	No	305.1	Unit/Enh (6490)	247.1	Unit/Enh (6490)	110	36	4	4.2	3
EIS	13C6-6-PPD-Quinone	No	305.1	Unit/Enh (6490)	221.1	Unit/Enh (6490)	110	20	4	4.2	3
Target	6-PPD-Quinone	No	299.1	Unit/Enh (6490)	241.1	Unit/Enh (6490)	105	32	4	4.2	3
Target	6-PPD-Quinone	No	299.1	Unit/Enh (6490)	215.1	Unit/Enh (6490)	105	16	5	4.2	3
Target	6-PPD-Quinone	No	299.1	Unit/Enh (6490)	187.1	Unit/Enh (6490)	105	32	5	4.2	3
IS	D5-6-PPD-Quinone	Yes	304.1	Unit/Enh (6490)	246.1	Unit/Enh (6490)	110	36	4	4.2	3
IS	D5-6-PPD-Quinone	Yes	304.1	Unit/Enh (6490)	220.1	Unit/Enh (6490)	110	20	4	4.2	3
IS	D5-6-PPD-Quinone	Yes	304.1	Unit/Enh (6490)	192.1	Unit/Enh (6490)	110	36	4	4.2	3

Source Parameters

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

Scan Parameters

Data Stg	Threshold
Centroid	0

Chromatograms

Chrom Type	Label	Offset	Y-Range
TIC	TIC	0	1500000

Instrument Curves

Actual

Name: Multisampler **Module:** G7167B

Report generation date: 06-Feb-2025 11:35:33 AM Page 2 of 7

Sampling Speed

Draw Speed	200.0 µL/min
Eject Speed	400.0 µL/min
Wait Time After Drawing	2.0 s

Injection

Needle Wash Mode	Multi-wash
Injection Volume	5.00 µL

Multi-wash

	Step	Solvent	Wash Time (s)	Seat Back Flush	Needle Wash	Comment
1	1	S1	5	Yes	Yes	
2	2	S2	5	Yes	Yes	
3	3	Off				
4	Start Cond.	S1		Yes	No	

High Throughput

Injection Valve to Bypass for Delay Volume Reduction	No
Sample Flush-Out Factor	5.0

Overlapped Injection

Overlap Injection Enabled	No

Needle Height Position

Draw Position Offset	0.0 mm
Use Vial/Well Bottom Sensing	No

Stoptime

Stoptime Mode	As Pump/No Limit

Posttime

Posttime Mode	Off

Pretreatment

	Function	Parameter
1	Draw	Draw 1.00 µL from location "Via 1" with default speed using default offset
2	Wash	Wash needle as defined in method
3	Draw	Draw default volume from sample with default speed using default offset
4	Wash	Wash needle as defined in method
5	Inject	Inject

Name: Binary Pump **Module: G7120A**

Flow	0.400 mL/min
Use Solvent Types	Yes
Stroke Mode	Synchronized
Low Pressure Limit	0.00 bar
High Pressure Limit	1000.00 bar
Max. Flow Ramp Up	100.000 mL/min²
Max. Flow Ramp Down	100.000 mL/min²
Expected Mixer	Jet Weaver V35 Mixer

Stroke A

Automatic Stroke Calculation A	Yes

Stoptime

Stoptime Mode	Time set
Stoptime	5.00 min

Posttime

Posttime Mode	Time set
Posttime	1.00 min

Solvent Composition

Acquisition Method Report

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	Channel	Ch. 1 Solv.	Name 1	Ch. 2 Solv.	Name 2	Selected	Used	Percent (%)
1	A	100.0 % Acetonitrile V.03		Organic	ACN 0.1% Formic	Ch. 2	Yes	30.00 %
2	B	100.0 % Water V.03		100.0 % Water V.03	H2O 0.1% Formic Acid	Ch. 2	Yes	70.00 %

Timetable

	Time (min)	A (%)	B (%)	Flow (mL/min)
1	Start. Cond. min	30.00 %	70.00 %	0.400 mL/min
2	0.30 min	30.00 %	70.00 %	0.400 mL/min
3	3.00 min	80.00 %	20.00 %	0.400 mL/min
4	4.00 min	95.00 %	5.00 %	0.400 mL/min
5	5.00 min	100.00 %	0.00 %	0.400 mL/min

Name: Column Comp. **Module: G7116B**

Left Temperature Control	
Temperature Control Mode	Not Controlled
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	
Right temperature Control Mode	Temperature Set
Right temperature	40.0 °C
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	
Enforce column for run enabled	No
Stoptime	
Stoptime Mode	As Pump/Injector
Posttime	
Posttime Mode	Off
Timetable	
Valve Position	Use current
Ready when front door open	No
Position Switch After Run	Do not switch

Name: VWD **Module: G7114B**

Analog Output	
Analog Zero Offset	5 %
Analog Attenuation	1000 mAU
Signals	
Signal	
Acquire Signal	No
Signal ID	Signal A
Signal Wavelength	250 nm
Autobalance	
Autobalance Prerun	Yes
Autobalance Postrun	No
Scan Variables	
Scan Range From	190 nm
Scan Range To	400 nm
Scan Range Step	2 nm
Dual-Wavelength settings	
Dual-Wavelength enabled	No
Stoptime	