FLORIDA DEPARTMENT OF ENVIRONMENTAL PROTECTION

DETERMINATION OF PETROLEUM-RANGE ORGANICS

Method FL-PRO

2018

1. Introduction

Method FL-PRO provides a method-defined measurement of petroleum concentration in environmental and waste samples in the C_8 – C_{40} hydrocarbon range (diesel through motor oils). Analysts must be experienced in the use of a gas chromatograph and skilled in the interpretation of gas chromatograms of petroleum hydrocarbons. All regulatory requirements for demonstration of analytical capability must be met before using FL-PRO to report sample analysis data required by the rules of the Florida Department of Environmental Protection (DEP).

2. Scope and Application

This method may not be used as an indication of gasoline contamination for purposes of demonstrating compliance with DEP rules. Method FL-PRO may be used to achieve the following measurement objectives.

2.1 Sample Matrices

Method FL-PRO may be used to extract and analyze water, soil, sediment and waste samples for petroleum hydrocarbons. See the recommended sample preparation methods in section 10 below.

2.2 Method Detection Limit (MDL) and Practical Quantitation Limit (PQL)

The laboratory must establish appropriate MDLs and PQLs for each matrix and sample type analyzed. Laboratories certified by the Florida Department of Health Environmental Laboratory Certification Program (ELCP) must determine and verify appropriate MDLs and Limits of Quantitation (LOQs or PQLs) according to all certification requirements and accreditation standards (NELAC and/or TNI, as applicable; see Reference 6) for each matrix for which the laboratory is certified or seeking certification for the FL-PRO method. Ensure that all steps in the sample processing and analysis procedures are included in the determination and verification of MDLs and PQLs.

2.2.1 The MDL for the FL-PRO method is highly dependent on the sample matrix, sample preparation procedures used, and the performance of the individual laboratory. Determine laboratory MDLs according to requirements specified in DEP rules, if applicable. If the MDL procedure is not specified by DEP rule, determine and verify the MDL using any technically justifiable and scientifically sound method appropriate for the FL-PRO procedures employed. DEP recommends using the EPA procedure (Reference 18) if an MDL procedure is not specified by rule.

2.2.2 Determine and verify the PQL using a defined procedure and criteria. For example, the PQL may be chosen to be the lowest calibration standard used for a calibration curve. Alternatively, the PQL may be indicated as the concentration at which a specific sample preparation method has been demonstrated to achieve an analytically determined range of precision and accuracy for a given matrix or sample type. When laboratory certification is not applicable as in 2.2 above, the PQL may be determined and verified by any technically justifiable and scientifically sound method appropriate for the FL-PRO procedures used. In all cases, the claimed PQL must correspond to a concentration at or above the lowest calibration standard used.

2.3 Linear Range

The linear (working) range of the FL-PRO method is dependent in part upon column type, detector sensitivity and injection volume. Typically, the approximate range for the FL-PRO method is 170 - 17,000 ng on-column of Total Petroleum Hydrocarbon Standard or PHS (see 8.4.3) and should be defined by the selection of calibration standard concentrations used for the initial calibration curve. Dilutions of samples must be performed as necessary to put the chromatographic envelope within the linear range of the method.

3. Summary of Method

Use the FL-PRO method to analyze samples for Petroleum Range Organic (PRO) compounds.

3.1 A measured amount of sample to be analyzed for PRO is spiked with at least two surrogate compounds and extracted with methylene chloride (dichloromethane). The extract is dried, concentrated, and treated with silica gel to remove potential organic interferences. The typical extract volume is 1 mL - 2 mL. An aliquot of the extract is injected onto a capillary column gas chromatograph (GC) equipped with a flame ionization detector (FID). Quantitation is based on the detector response compared to a series of even-numbered normal alkane standards.

3.2 The FL-PRO method is based in part on the information found in the references listed in section 14, below.

4. Definitions

The following terms and descriptions of requirements are applicable to the FL-PRO method procedures.

4.1 Initial Calibration Verification (ICV)

All initial calibrations must be verified with a standard obtained from a second manufacturer or a separate lot prepared independently by the same manufacturer. Traceable standards should be used, if available.

4.2 Laboratory Control Sample (LCS)

The LCS (however named) may be a laboratory fortified blank, spiked blank, reference sample, or QC check sample, prepared in a clean sample matrix, such as analyte-free water, as described in 8.1, or sand, soil or other quality control matrix, free from petroleum hydrocarbons

and interferences (see 4.4 for non-aqueous matrix for method blanks). The LCS is spiked with the Petroleum Hydrocarbon Standard and taken through all sample preparation and analytical steps of the FL-PRO method. The LCS is used to control the accuracy of the method and may also be used to control the precision of the method if LCS duplicates are prepared and analyzed.

4.3 Matrix Spike (spiked sample or fortified sample)

A field sample composed for each preparation batch by adding a known amount of the Petroleum Hydrocarbon Standard and taken through all sample preparation and analytical steps of the FL-PRO method. Matrix spikes are used to determine the effect of the field sample matrix on analyte recovery.

4.4 Method Blank

A sample of a clean matrix similar to the batch of associated samples (when available) that is free from the analytes of interest and is processed simultaneously with and under the same conditions as samples through all steps of the preparation and analytical procedures, and in which no target analytes or interferences are present at concentrations detected at or above the laboratory MDL. A method blank may be prepared using analyte-free water (8.1) for aqueous samples, or clean sand (e.g. Ottawa sand) or sodium sulfate (8.3) for soil samples.

4.5 Petroleum Hydrocarbons

All resolved and unresolved chromatographic peaks, eluting between just before the peak start of n-octane (n-C_8) and just after the peak end after n-tetracontane (n-C_{40}). Quantitation is based on direct comparison of the area within this range to the total area of the Petroleum Hydrocarbon Standard (PHS) as determined from the FID detector response, using baseline-to-baseline integration. The quantified amount contributed by the surrogate peaks eluting within this range is subtracted from the PHS amount in the final calculation.

4.6 Petroleum Hydrocarbon Standard (PHS)

A seventeen-component mix of all even-numbered normal alkanes from C_8 to C_{40} (Table 1). This standard is used for quantitation and to determine retention time windows and the integration time interval for the resolved and unresolved complex mixture of petroleum hydrocarbons reported as FL-PRO.

5. Interferences

5.1 Other organic compounds, including chlorinated hydrocarbons, phenols, and phthalate esters, as well as biodiesel fuels, are measurable by the FL-PRO method as constituents of the PRO. Biodiesel fuels will typically elute shortly after the *o*-terphenyl surrogate as a cluster of several sharp peaks.

5.2 Animal and vegetable oil and grease may also be partially measurable as part of the PRO (C8 to C40) integration if the sample is not cleaned up before analysis. Peaks that typically elute include minor impurities or decomposition products. Animal fats and vegetable oils are mixtures of fatty acid triglycerides, which have much higher boiling points than PRO constituents. To eliminate false positives from these sources, the silica cleanup of extracts (10.6) is a mandatory part of

sample preparation when using any of the sample preparation procedure options specified in this method. However, depending on the amount of silica gel and the cleanup technique used, there is still a possibility that large concentrations of fats and oils may be retained in the final extract after cleanup.

5.3 A variety of biogenic materials may also be extracted by the sample preparation procedures available for the FL-PRO method. This includes various terpenes as well as plant oils and waxes that are often present in vegetation-rich soils. Other natural organic compounds that may be measured are long chain carboxylic acids (fatty acids), and steroids such as cholesterol. Lastly, not all petroleum range hydrocarbons originate from petroleum products. Some background levels of various hydrocarbons are to be expected in certain organic-rich formations like peat moss or other accumulations of partially decayed vegetation.

5.4 High purity reagents (pesticide grade or better) must be used to minimize interference problems.

5.5 The likelihood of certain method interferences is reduced by washing all glassware and other reusable labware with hot water and detergent solution, followed by successive rinses with tap water or analyte-free water (8.1), acetone, and methylene chloride. At least one method blank must be analyzed with each preparation batch or for every 20 prepared samples to monitor for contamination and interferences.

5.6 Contamination by carryover can occur whenever high-level and low-level samples are sequentially analyzed. Whenever an unusually concentrated sample (or standard) is analyzed, it should be followed by an analysis of a solvent blank to check for instrument contamination, prior to running additional samples in the analytical sequence.

6. Safe Handling of Chemicals

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. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. Maintaining a current awareness file of Occupational Safety and Health Administration (OSHA) regulations regarding the safe handling of the chemicals specified in this method is recommended, and a reference file of Material Safety Data Sheets (MSDS) should be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety should be available and be identified for use by the analyst.

7. Apparatus

The following equipment is recommended for the detailed procedures described in this method. Other apparatus may be used where specifications for the equipment are equivalent or exceed those listed. For additional guidance on equipment and supply selection, see the relevant methods in SW-846 (Reference 15). Alternative equipment used for approved modifications to the FL-PRO method described in Section 12 are also acceptable.

7.1 Glassware

All glassware specifications are suggested only and are dependent on procedures employed.

7.1.1 Glass separatory funnel, 2000 mL with Teflon® stopcock.

7.1.2 Concentrator tube, Kuderna-Danish (K-D), 10 mL graduated (Kontes, or equivalent). Calibration must be checked at the volumes employed in the test. Ground glass stopper is used to prevent evaporation of extracts.

7.1.3 Evaporative flask, Kuderna-Danish, 500 mL (Kontes, or equivalent). Attach to concentrator tube with springs or other appropriate attachment devices.

7.1.4 Snyder column, Kuderna-Danish, three-ball, macro (Kontes, or equivalent).

7.1.5 Other extraction concentration procedures such as a rotary evaporation system, nitrogen evaporators, or automated evaporation systems such as TurboVap® may also be used.

7.1.6 Vials, amber glass, 10 to 15 mL capacity, with Teflon®-lined screw cap; or, glass vials with Teflon®-lined cap suitable for use with a GC auto-sampler.

7.1.7 Disposable glass pipets, Pasteur-type.

7.1.8 Glass beakers, 400 mL, 250 mL.

7.1.9 Drying column, 20 mm I.D., Pyrex chromatographic column, with precleaned, silanized glass wool at bottom, and Teflon® stopcock.

7.1.9.1 Note: Fritted glass discs are difficult to decontaminate after highly contaminated extracts have been passed through.

7.1.9.2 Columns without frits may be purchased.

7.1.9.3 Use a small pad of precleaned, silanized glass wool to retain the absorbent.

7.1.9.4 Prewash the glass wool pad with 50 mL of acetone followed by 50 mL of methylene chloride prior to packing the column with absorbent.

7.2 Boiling chips, approximately 10/40 mesh. Heat to 400º for 30 minutes or extract with methylene chloride in a Soxhlet extractor.

7.3 Glass microsyringes, 1µL, 5 µL, 10 µL, 25 µL, and 100 µL capacities.

7.4 Water bath, heated, with concentric ring cover, capable of temperature control (± 2º C). The bath should be used in a hood.

7.5 An analytical balance capable of accurately weighing 0.0001 g should be used for standards. A top-loading balance capable of weighing to the nearest 0.1 g should be used for sample preparation.

7.6 Gas Chromatography

Ensure the gas chromatography system for the FL-PRO method meets the following general specifications. The columns recommended below may be substituted with alternative columns that will allow the performance criteria of the method to be met.

7.6.1 Gas Chromatograph

Analytical system complete with a gas chromatograph and all required accessories, including gas flow controllers, Flame Ionization Detector, column supplies, gases, and stainless-steel diaphragm gas regulators. A data system capable of determining peak areas using a forced baseline and baseline projection is required. A data system capable of storing and reintegrating chromatographic data is highly recommended.

7.6.2 Columns

Other GC columns may be used if all performance criteria in this method can be met. Capillary columns are required to achieve the necessary resolution. Megabore columns may be acceptable. The column must resolve typical petroleum components and resolve the solvent front from C_8 (*n*-octane).

- Column 1: 30 M X 0.25 mm DB-5 or equivalent, minimum of 0.25 micron film thickness.
- Column 2: 30 M X 0.25 mm DB-17 or equivalent, minimum of 0.25 micron film thickness.

7.7 Sonication

Use a horn-type sonicator equipped with a titanium tip (Qsonica, or equivalent) for preparation of soils and other applicable non-aqueous samples.

7.7.1 Power wattage must be a minimum of 375W, with pulsing capability.

7.7.2 Use accessory 1/2" and 3/4” tapped disrupter horns, and a 1/8" standard tapered microtip probe.

7.7.3 A sound enclosure is recommended with the above disrupter for decreasing sound (Qsonica or equivalent).

7.8 Vacuum filtration apparatus.

7.9 Buchner funnel.

7.10 Filter paper, Whatman No. 41 or equivalent.

7.11 Soxhlet Extraction

Soxhlet apparatus and supplies may include the following items.

7.11.1 Soxhlet extractor, 40 mm ID, with 500 mL round bottom flask.

7.11.2 Glass or paper thimble and/or contaminant-free, precleaned, silanized glass wool.

7.11.3 Heating mantle, rheostat-controlled.

7.12 Percent Moisture Determination

Moisture determination apparatus and supplies may include the following items.

7.12.1 Drying oven capable of maintaining 105 ºC.

7.12.2 Desiccator.

7.12.3 Crucibles, porcelain or disposable aluminum.

8. Reagents and Standards

The following reagents and standards are required or recommended for specific procedures described in FL-PRO.

8.1 Analyte-free water

Carbon-filtered deionized water or other reagent water shown to be free of all interfering components. Use for glassware and labware cleaning, dilutions, method blanks, and laboratory control spikes.

8.2 Solvents

Acetone, carbon disulfide, chloroform, hexane, methanol, methylene chloride (pesticide grade or equivalent).

8.3 Sodium sulfate, granular, anhydrous (ACS)

Purify by heating at 400 ºC for 4 hours in a shallow tray, or by extracting 3 times with methylene chloride and drying at 105 ºC.

8.4 Stock standard solutions

Prepare the following stock standards. Unless noted, prepare all standards in methylene chloride.

8.4.1 Surrogate Standards, prepared at a recommended stock solution concentration of 5000 µg/mL (mg/L):

- *Ortho*-terphenyl (OTP, C_{19}), in methanol
- Nonatriacontane (C_{39}), in carbon disulfide

8.4.1.1 Suggested solvents for diluted surrogate standards are acetone, methanol or carbon disulfide, depending on the solubility of concentrated solutions. Solvent mixtures such as 1:1 acetone/hexane may also be used.

8.4.1.2 Surrogate spiking concentrations should be appropriate for the concentration range of field samples, quality control samples and standards analyzed. DEP recommends spiking all analyzed standards and samples in the range of 50 – 200 mg/L (or mg/kg for non-aqueous analyses), or in the middle of the calibration range. Other surrogate concentrations may be used for spiking samples for MDL studies (2.2). A recommended stock surrogate spiking solution concentration is 100 μg/mL in 1:1 acetone/hexane for both surrogates in 8.4.1 above.

8.4.1.3 When using prepared solutions of C_{39} surrogate standard, observe the following precautions to mitigate problems with the solubility of this compound at higher concentrations:

(a) Visually check solutions for precipitation before withdrawing aliquots or injecting standards.

(b) Warm refrigerated solutions of C_{39} to room temperature before use. Mix warmed solutions by sonication and manually shaking (or use a vortex mixer).

(c) Repeat steps (a) and (b) above any time aliquots of C_{39} standard solutions or spiked samples are taken for portioning into other volumes.

(d) Ensure transfers of aliquots are quantitative by monitoring good transfer technique.

8.4.1.4 A calibration curve for surrogate standards is recommended (but not required), and may be constructed from the responses to increasing concentrations of surrogates added to the corresponding calibration standard solutions.

8.4.1.5 An appropriate volume of surrogate spiking solution must be added to all samples, standards, and quality control samples before extraction. See section 10.

8.4.2 Individual stock solutions of C_8 - C_{40} even normal alkanes (see Table 1):

For solubility reasons, it may be necessary to prepare stock solutions of *n*-alkanes in other solvents such as hexane, chloroform, acetone, or carbon disulfide. Traceable standards should be obtained from vendors when available.

8.4.3 Petroleum Hydrocarbon Standard (PHS)

DEP recommends preparing a stock solution of even-numbered normal alkane standards (C_8 - C_{40}) with each component at 500 μg/mL.

8.4.3.1 Working-level PHS Calibration Standards

Suggested concentrations for PHS calibration standards are 5.0, 25, 50, 100, 150, 200, and 300 μg/mL (mg/L) of each individual component. This is equivalent to 85, 425, 850, 1700, 2550, 3400, and 5100 μg/mL total alkanes in the standards, and is defined as Total PHS.

8.4.3.2 Other calibration levels may be used depending on the expected concentration of PRO (as defined by Total PHS) in field samples.

8.4.3.3 It is recommended that the OTP and C_{39} surrogate standards should each be spiked at a concentration of 100 μg/mL to determine initial surrogate responses, in a mid-level calibration standard. If a calibration curve for the surrogates is constructed, other calibration standards should have the surrogates added at levels following the pattern of concentration ratios for Total PHS (see 8.4.1.2 and 8.4.3.1).

8.4.3.4 Second-source PHS Standards

All initial calibrations must be verified with a PHS solution made from *n*-alkane standards obtained from a second manufacturer or a separate lot prepared independently by the same manufacturer. Traceable standards should be used, if available.

8.4.4 Stock PHS spike solution

Prepare a working solution of Petroleum Hydrocarbon Standard in acetone designed to provide a Total PHS concentration in spiked samples at a level that is appropriate for expected background PHS concentrations in the analyzed field samples.

8.4.5 Pattern Recognition Standards

Various commercial petroleum standard solutions or products can be used by the laboratory to identify petroleum product types. These may be purchased from vendors or obtained from commercial sources.

8.4.6 Silica gel

Bulk reagent or cartridge silica gel products may be used.

- Silica gel solid-phase extraction cartridges, 40 μm particles, 60 Angstrom pores; 0.5 g or 1g (Restek, Supelco, or equivalent).

- Silica gel, 60 to 200 mesh, deactivated with 1% - 2% water (Davisil, Fisher or equivalent).

9. Sample Collection, Preservation, Containers, and Holding Times

The requirements in DEP SOP FS 1000, tables FS 1000-5 and FS 1000-6 (DEP-SOP-001/01, January 2017, Reference 17) list the mandatory criteria for sample preservation, sample containers and sample holding times from collection to analysis for aqueous and non-aqueous FL-PRO samples, respectively. The collection of FL-PRO samples must follow all applicable DEP SOP requirements. The following is a summary of the criteria and requirements.

9.1 Whenever possible, samples must be collected as discrete grab samples, which are collected directly into the sample container. Sample collection equipment such as bailers or intermediate containers must be avoided unless needed for collection from monitoring wells or surface water at depth. Unless required by permit, automatic samplers may not be used. Pumps

such as bladder pumps should not be used, but peristaltic pumps may be used with disposable tubing, if necessary to access the sampling point.

9.2 All sampling equipment which contacts the sample must be constructed of Teflon®, stainless steel, glass, polypropylene, or polyethylene. Do not used flexible PVC tubing such as Tygon® for the purging or sample collection process.

9.3 Water samples should be collected in a one-liter glass container; soils and wastes in a glass jar. Alternative sample volumes may be collected if DEP data quality objectives are achieved using the different volumes (see section 11). All containers must be sealed with a screw cap with Teflon® liner. Water samples must be acidified to a pH of less than 2 with hydrochloric or sulfuric acid (ACS reagent grade or better).

9.4 The samples must be stored at ≤ 6 ºC from the time of collection until extraction. Do not freeze samples.

9.4.1 Samples hand-delivered to the laboratory on the day of sample collection and received in wet ice may not have equilibrated to the required preservation temperature by the time of receipt.

9.4.2 Extraction must be performed on waters within 7 days of sample collection and on soils and wastes within 14 days of sample collection. Sample extracts must be stored at ≤ 6 ºC. All analyses must take place within 40 days of extraction.

10. Procedure

The FL-PRO method of analysis uses the syringe injection of solvent extracts of aqueous samples or extracts of soils, sediments and wastes, utilizing gas chromatography with FID.

10.1 Sample preparation - Introduction

The FL-PRO sample preparation methods are based on EPA SW-846 Methods 3510C (Separatory Funnel Liquid-Liquid Extraction) and 3520C (Continuous Liquid-Liquid Extraction) for water; 3540C (Soxhlet Extraction) and 3550C (Sonication) for soils, sediments and certain wastes; and, 3580A (Waste Dilution) for soluble wastes.

10.1.1 Modifications to the liquid-liquid extraction procedure (including methods based on EPA 3511), that reduce sample volume and/or final volume of extract, are generally acceptable (see section 11).

10.1.2 Modifications to sample preparation procedures based on, for example, EPA SW-846 Methods 3535A, 3541, 3545A, 3546, 3560, 3570, and 3630C may also be used.

10.1.3 Modified procedures must allow achievement of the QC criteria and requirements in this method, and meet additional requirements as described in section 11 of this method.

10.1.4 Regardless of the preparation method used, the use of surrogates and a silica gel clean-up of the extract is mandatory for proper sample preparation and quality control.

10.2 Water Extraction - Separatory Funnel

Extract one liter of water sample for separatory funnel extraction using the following procedure.

10.2.1 For later determination of the extracted sample volume, weigh each sample and container before pouring out the sample for extraction.

10.2.2 Pour the sample into a 2-liter separatory funnel. For blanks and laboratory quality control standards, pour 1 liter of analyte-free water (8.1) for each quality control sample into separatory funnels.

10.2.3 Determine extracted sample volumes by weighing empty sample containers after extraction and subtracting the empty weight from the total weight measured in 10.2.1. Record all measured weights and volume determinations.

10.2.4 Check and note the initial pH of the sample.

10.2.5 Add 2 mL each of OTP and C_{39} surrogate standard at 100 µg/mL to all samples, spiked samples, duplicates, method reagent blanks and laboratory control samples (see 8.4.1). Other surrogate concentrations may be used for spiking samples for MDL studies (2.2). Add PHS spike mixture (8.4.4) to selected samples and laboratory control samples.

10.2.6 Prepare at least one method reagent blank, one sample matrix spike and either a matrix spike duplicate or duplicate sample, with each batch of 20 samples or less. Laboratory control samples must be added as a minimum quality control measure of accuracy.

10.2.7 Add 60 mL methylene chloride to the sample bottle and rotate the bottle to rinse the sides. Transfer the solvent to the separatory funnel. Extract the sample by shaking it for two minutes with frequent ventilation.

10.2.8 Allow the organic layer to separate from the water phase for a minimum of 10 minutes.

10.2.9 If an emulsion interface between layers is more than one-third the size of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. The optimum technique depends upon the sample and may include stirring, pressure or vacuum filtration of the emulsion through precleaned, silanized glass wool, centrifugation, or other physical methods. If the emulsion cannot be broken (recovery of 80% of the methylene chloride, corrected for the water solubility of methylene chloride), transfer the sample, solvent, and emulsion into the extraction chamber of a continuous extractor and proceed as described in Section 10.3

10.2.10 Drain the bottom layer (methylene chloride) into a 250 mL beaker or flask.

10.2.11 Repeat the extraction twice more using a 60 mL aliquot of methylene chloride each time. Collect the solvent in the same container described in 10.2.10. Record the volume recovered.

10.2.12 Dry the extract by passing it through a drying column containing about 10 cm of anhydrous sodium sulfate. Collect the dried extract in a Kuderna-Danish (K-D) concentrator assembly. Rinse beaker or flask, which contained the solvent extract, with 20-30 mL of

methylene chloride and add it to the column to complete the quantitative transfer.

10.2.13 Alternatively, put a plug of precleaned, silanized glass wool in a funnel and fill about two thirds of the funnel with sodium sulfate. Rinse the funnel and sodium sulfate with 30-40 mL of methylene chloride and discard the rinse solvent appropriately. Pour the sample extract through the sodium sulfate into a 500 mL K-D evaporative concentrator assembly. Rinse the beaker and the sodium sulfate with small amounts of methylene chloride. Add these rinses to the K-D concentrator.

10.2.14 Add a boiling chip to the K-D concentrator and attach a 3- ball Snyder column to the top. Pre-wet the column by adding about 1 mL of Methylene chloride to the top. Note: The concentration step is critical. Losses of analytes can occur if care is not taken in processing the extracts and performing all quantitative transfers of extracts between vessels and containers.

10.2.15 Place the K-D apparatus in a hot water bath (60 – 65 ºC) so that the concentrator tube is partially immersed in the hot water and the entire lower rounded surface of the flask is bathed with hot vapor. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 10 -20 minutes. At the proper rate of distillation, the balls of the column will actively chatter, but the chambers will not flood.

10.2.16 When the apparent volume of liquid reaches 1 mL, remove the K-D apparatus from the water bath and allow it to drain and cool for at least 10 minutes. When the volume is reduced below 1 mL, semivolatile analytes may be lost.

10.2.17 If the extract is highly colored or a precipitate forms during concentration, the final volume should be higher (5-10 mL).

10.2.18 After the K-D has cooled, rinse the Snyder column and middle flask with a small amount of methylene chloride. Be sure to rinse all the ground glass joints well, as compounds collect on the ground glass. Final volume should be 2 mL. If it is greater than 2 mL, proceed to 10.2.19. If it is 2 mL, proceed to 10.2.20.

10.2.19 If further concentration is required, add another clean boiling chip to the concentrator tube and attach a 2-ball micro-Snyder column. Prewet the column by adding 0.5 mL of methylene chloride or exchange solvent to the top of the column. Place the K-D apparatus in a hot water bath so that the concentrator tube is partially immersed in the hot water. Adjust the vertical position of the apparatus and the water temperature, as required, to complete the concentration in 5-10 minutes. At the proper rate of distillation, the balls of the column will actively chatter, but the chambers will not flood.

10.2.19.1 When the apparent volume of liquid reaches 0.5 mL, remove the K-D apparatus from the water bath and allow it to drain and cool for at least 10 minutes.

10.2.19.2 Remove the Snyder column and rinse the flask and its lower joints in the concentrator tube with 0.2 mL of extraction solvent. Adjust the final volume to 2.0 mL.

10.2.20 Alternatively, concentrate the extract to 2.0 mL under a gentle stream of nitrogen using a nitrogen evaporator apparatus. If the extract is highly colored, forms a precipitate, or stops evaporating, the final volume should be higher (5-10 mL).

10.2.21 Automated evaporator systems such as TurboVap® may also be used.

10.2.22 Transfer the extract to a labeled 4 mL (or 12 mL) vial with Teflon®-lined cap, mark the meniscus and record the final volume.

10.2.23 Record the prep information for the extraction and concentration steps.

10.2.24 Proceed to 10.6 for cleanup.

10.3 Water Extraction - Continuous Liquid-Liquid Extraction

See EPA SW-846 method 3520C for additional guidance (Reference 15).

10.3.1 Mount the continuous extractor on appropriate racks.

10.3.2 Pour 250 mL of methylene chloride in a round bottom flask, add a few boiling chips. Add 300 mL of methylene chloride to the extractor flask.

10.3.3 When pouring water into the extractor, minimize the disturbance of the solvent layer and avoid getting water into either sidearm by pouring the water down the back of the extractor.

10.3.4 Check and note the sample pH.

10.3.5 Weigh the sample and container prior to pouring for extraction as in 10.2.1. Pour the sample into the continuous liquid-liquid extractor. For blanks and laboratory control samples, use 1 liter of analyte-free water (8.1) for each quality control sample.

10.3.6 Add 2 mL each of OTP and C_{39} surrogate standard surrogate at 100 µg/mL to all samples, spiked samples, duplicates, method blanks and laboratory control samples (see 8.4.1). Other surrogate concentrations may be used for spiking samples for MDL studies (2.2). Add PHS spike mixture (8.4.4) to selected samples and laboratory control samples.

10.3.7 Prepare at least one method blank, one sample matrix spike and either a matrix-spike duplicate or sample duplicate, with each batch of 20 samples or less. Laboratory control samples must be included as the minimum quality control measure for accuracy.

10.3.8 Add enough analyte-free water to the extractor flask to allow the solvent in the removable sidearm to just begin to drip into the round bottom flask.

10.3.9 Remove the condenser from the rack and wipe the lower joint lip with a laboratory tissue soaked with methylene chloride. Place the condenser on the top of the extractor. Turn on the cool water supply and check the flow indicators.

10.3.10 Turn on the heating mantle. Record the starting time on the prep sheet. Check after 15 minutes to be sure that the solvent in the round bottom flask is boiling, that solvent is dripping from the lip on the condenser, and that the volume of the solvent in the round bottom flask is still about 240 mL.

10.3.11 Check all extractor joints for leaks with a laboratory tissue. Allow the extraction to

proceed for 18-24 hours.

10.3.12 Turn off the heating mantle and allow the apparatus to cool (30 - 60 minutes) with water flowing through the condenser.

10.3.13 The solvent contained in the round bottom flask is the extract. Transfer the extract to a 400-mL beaker, rinsing with a small amount of methylene chloride. If the volume of solvent is less than about 250 mL, record the solvent volume.

10.3.14 Determine and record the extracted sample volume as in 10.2.3.

10.3.15 Go to 10.2.12 and continue with the extract preparation.

10.4 Soil Preparation - Sonication

See EPA SW-846 method 3550C for additional guidance (Reference 15).

10.4.1 Note the apparent condition of the sample, especially observations that may influence the interpretation of analysis results (e.g. presence of foreign materials, variable particle size, presence of sheen). Remove large rocks or other foreign materials (e.g., glass, metal, etc.) and mix the sample well.

10.4.2 Since sample concentrations must be reported as mg/kg dry weight, determine sample moisture content as follows.

10.4.2.1 Pre-weigh an aluminum weighing boat.

10.4.2.2 Weigh 5-10 g of the sample into the boat and record the weight of the boat and the combined weight of the boat and sample.

10.4.2.3 Dry the sample in a hood or warm to no greater than 100 ºC ± 5 ºC in a drying oven overnight. **Note**: **Make sure the drying oven is placed under a hood. Heavily contaminated soils will produce strong organic vapors which may be detrimental to analysts' health.**

10.4.2.4 Cool in a desiccator until the sample reaches room temperature and weigh for the moisture determination using the below formula.

% Moisture = (A-C)/(A-B) x 100

Where:

A = weight of boat + wet sample

B = weight of boat

C = weight of boat + dry sample

10.4.3 Weigh a 25 g aliquot of the original sample into a 400 mL beaker or a 250 mL centrifuge bottle (do not use the dried sample from 10.4.2). Record weight to the nearest

0.1 g.

10.4.4 Prepare at least one method blank, one sample matrix spike and either a matrix spike duplicate or a duplicate sample with each batch of 20 samples or less. Weigh QC sample aliquots as in 10.4.3. Laboratory control samples (e.g., Ottawa sand) must be included as a minimum quality control measure.

10.4.5 Add 2 mL each of 100 μg/mL OTP and C_{39} (8.4.1) to all samples and quality control samples. Add PHS spike mixture (8.4.4) to selected samples and laboratory control samples. Mix the samples immediately.

10.4.6 Add 25 g of dried sodium sulfate and stir the mixture well with a stainless-steel spatula. The sample should have a free-flowing sandy texture. If it forms a large clump, add more sodium sulfate until the proper texture is achieved and note on the prep sheet. The sodium sulfate may also serve as the clean matrix for the method blank and/or LCS.

10.4.7 Add 100 mL of methylene chloride to all prepared samples.

10.4.8 Sonicate the samples for 3 minutes at an output setting of 10, with the ¾-inch sonicator horn placed 1/2 inch below the surface of the solvent. The sonicator should be in the 1-second pulse mode, with the duty cycle set a 50%. Follow manufacturer recommendations for other sonicator models, as necessary. **Note**: **Depending on the sonication equipment, a setting of 10 may shatter beakers. If the sonication unit has a demonstrated history of these occurrences, the setting may be lowered to avoid glass breakage. The setting should not be lowered past 7.**

10.4.9 Decant and filter extracts through Whatman No. 41 filter paper using vacuum filtration or centrifuge and decant the extraction solvent.

10.4.10 Repeat the extraction two more times with two additional 100-mL portions of solvent. Decant off the extraction solvent after each sonication. On the final sonication, pour the entire sample into the Buchner funnel and rinse with extraction solvent.

10.4.11 Record the total volume of the solvent that is recovered.

10.4.12 Go to 10.2.12 and continue with the preparation.

10.5 Soil Preparation - Soxhlet Extraction

See EPA SW-846 method 3540C for additional guidance (Reference 15).

10.5.1 Proceed with 10.4.1 and 10.4.2.

10.5.2 Weigh a 10g aliquot of the sample to the nearest 0.1 g and transfer into the thimble (do not use the dried sample from 10.4.2).

10.5.3 Prepare at least one method blank, one sample matrix spike and either a matrix spike duplicate or a duplicate sample with each batch of 20 samples or less. Weigh QC sample aliquots as in 10.5.2. Laboratory control samples (e.g. Ottawa sand) must be included as a minimum quality control measure.

10.5.4 Add 2 mL each of 100 μg/mL OTP and C_{39} (8.4.1) to all samples and laboratory quality control samples.

10.5.5 Add PHS spike solution (8.4.4) to selected sample(s) and laboratory control samples. Mix samples immediately.

10.5.6 Blend 10 g of sodium sulfate with the sample. The sample should have a free-flowing, sandy texture. If the sample clumps, add more sodium sulfate until the proper texture is achieved and note the addition.

10.5.7 The extraction thimble must drain freely for the duration of the extraction period. Use precleaned, silanized glass wool plugs above and below the sample in the Soxhlet extractor as an acceptable alternative for the thimble.

10.5.8 Place loaded thimbles in extractors.

10.5.9 Add 300 mL of methylene chloride to the 500-mL extraction flask. Also add a few solvent-washed boiling chips to the flask. Connect the extractor.

10.5.10 Allow samples to extract for 18-24 hours, or as necessary to achieve optimum surrogate recovery at 4-6 cycles per hour. Be sure that coolant is flowing around the condensers.

10.5.11 Allow the extract to cool after the extraction is complete.

10.5.12 Go to 10.2.12 and continue with the preparation.

10.6 Silica Gel Cleanup (mandatory for all extracts)

10.6.1 Add 0.3 g of loose silica gel to the extract and shake the mixture for 5 minutes, or pass the extract through a 0.5g silica gel solid-phase extraction cartridge conditioned with 5 mL of methylene chloride.

10.6.2 When using an extraction cartridge, the recovered volume of solvent passing through the cartridge must be less than or equal to the recorded extract volume from 10.2.22. If it is not, the recovered volume must be used in calculations.

10.6.3 When using loose silica gel, filter the extract through a plug of precleaned silanized glass wool in a disposable glass pipette or allow to settle and decant extract into an appropriate vial.

10.6.4 Transfer cleaned extract to a labeled vial with a Teflon®-lined screw cap or sealed Teflon®-lined septum vial for analysis.

10.7 Dilution Technique

Use dilution to prepare petroleum products or waste samples which are soluble in methylene chloride.

10.7.1 Weigh 1 g of sample into a 10mL volumetric flask.

10.7.2 Add 2 mL each of 100 µg/mL OTP and C_{39} (8.4.1) to all samples and laboratory quality control samples.

10.7.3 Add PHS spike solution (8.4.4) to selected sample(s) and laboratory control samples. Mix samples immediately.

10.7.4 Dilute to 10 mL with methylene chloride.

10.7.5 Pass an aliquot of sample through a small (pipet) column of sodium sulfate to dry.

10.7.6 Proceed to 10.6 for silica gel cleanup.

10.8 Gas Chromatography (GC) Conditions

The recommended GC conditions are listed below. Chromatographic performance is demonstrated by resolution of standards and ability to model the response of the detector during calibration; also important are sensitivity, bias, and precision. For any chromatographic procedures or conditions used, the laboratory must demonstrate that the performance satisfies the analytical requirements and performance criteria of this FL-PRO method. See EPA SW-846 methods 8000D and 8015D for additional recommendations for optimizing chromatographic performance (Reference 15).

10.8.1 Recommended GC Conditions

Columns (7.6.2) and conditions can be modified if adequate performance is demonstrated per FL-PRO method requirements.

10.8.1.1 Set helium column pressure to 20 p.s.i. or at optimum pressure for column diameter.

10.8.1.2 Set column temperature to 40 ºC for 1 minute, then 10 ºC/min to 300 ºC and hold until the last standard (C_{40}) has eluted so that baseline quantitation is possible (run time averages approximately 60 minutes).

10.8.1.3 Set FID Detector to 300 ºC and injector to 280 ºC.

10.8.2 Performance Criteria

Choose GC run conditions and columns to meet the following criteria:

10.8.2.1 Ensure resolution of the C_8 peak from the solvent front. Micro-extraction methods frequently use large volume injection (LVI) techniques to achieve required Method Detection Limits (MDLs) or Practical Quantitation Limits (PQLs). Whenever “solvent vent” protocols are employed for LVI, ensure that the recoveries for the low-end hydrocarbons (especially C_8) are not adversely affected.

10.8.2.2 The column must be capable of separating typical petroleum hydrocarbon components from the surrogates.

10.9 Calibration

All samples must be associated with an acceptable initial calibration. Verify the calibration prior to sample measurements by analysis of initial and continuing calibration verification standards. Certified laboratories must ensure that all calibration requirements are met as required to maintain certification according to accreditation standards (i.e. NELAC or TNI standards, as applicable). For general information and recommendations about chromatography calibration, see EPA SW-846, method 8000D (Reference 15).

10.9.1 Initial Calibration:

Calibration must be by external calibration using a minimum of 5 concentration levels for the initial calibration.

10.9.1.1The standard concentrations must fall within the linear range of the curve (2.3).

10.9.1.2 The lowest standard must be at a concentration equal to or lower than the practical quantitation limit reported by the laboratory, and the highest concentration must quantitatively bracket the expected range of field sample concentrations.

10.9.1.3 Quantitation must be by calibration factor, linear regression, or non-linear regression. Refer to EPA SW-846 method 8000D, section 11.5 (Reference 15) for acceptance criteria and calculations for calibration model options.

10.9.1.4 In all cases, response of the standards must be determined by continuous integration of **all** responses (**excluding** surrogates) from a forced baseline beginning at a point prior to the elution of the C_8 peak to a point past the C_{40} peak. All responses must be determined as responses to baseline and not valley to valley. The selection of the time interval (i.e. time from beginning to end of integration) should be based on injection of PHS standards (8.4.3) and **must be constant for all standards and samples**. See 10.10 (Retention Time Window).

10.9.1.5 The criteria discussed below for evaluation of standard responses for calibration factors and calibration curves pertain to a linear calibration model. However, non-linear calibration curve regression can be used.

(a) Calibration Factor Calculation and Evaluation

Determine the total area response of the seventeen PHS components and calculate the calibration factor for each level as the ratio of area of standard mix against the mass injected:

$$\text{Calibration Factor} = \frac{\text{Total PHS Area}}{\text{Total PHS Mass Injected (ng)}}$$

(1) For the above formula:

Total PHS Area = Response as integrated in 10.9.1.4

Total PHS Mass Injected = The sum of the concentration of the individual components (8.4.3) multiplied by the volume injected (calculated as ng)

(2) If the percent relative standard deviation of the calibration factor is less than or equal to 20% over the working range, the linearity through the origin can be assumed and the average calibration factor can be used in place of a calibration curve.

(b) Calibration Curve Regression Evaluation

When using the average calibration factor (10.9.1.6) in lieu of a calibration curve regression, the percent relative standard deviation (RSD) for the average calibration factor must be ≤20% to demonstrate linearity through the origin. If the RSD for the average calibration factor exceeds 20%, choose a linear or non-linear regression model for the calibration. Calculate acceptance criteria for a calibration curve regression using the total PHS area (10.9.1.4) versus the PHS concentration (8.4.3). If a linear regression is applied to the calibration response, the correlation coefficient (r) must be equal to or greater than 0.995. For an acceptable non-linear regression, the coefficient of determination (r^2) must be ≥ 0.990.

10.9.1.6 Other calibration models may be used per guidance in EPA SW-846 methods 8000D and 8015D (Reference 15). Evaluate and verify all calibrations as below.

10.9.1.7 Initial Calibration Verification:

The accuracy of the initial calibration must be verified by injecting an Initial Calibration Verification standard (ICV) at a midpoint concentration level. An ICV standard is a standard mix that has been obtained from a different source (i.e., different lot or vendor). Traceable standards should be used, if available.

(a) Evaluation of the ICV

The calculated value must be within ±25% of the expected value. If an ICV failure is determined to have only impacted the ICV result and no samples have been analyzed, analyze another ICV and evaluate the result. If the second ICV result is acceptable, proceed with sample analysis. Document the cause of the ICV failure and reanalysis.

(b) Acceptance of Initial Calibration

If the initial calibration fails any of the criteria specified in sections 10.9.1.6 – 10.9.1.7 (a) above, perform any necessary corrective actions, analyze a new calibration curve, and evaluate the initial calibration for acceptance prior to analyzing any samples.

10.9.2 Continuing Calibration Verification:

The working calibration factor or calibration curve must be verified at the beginning of each 12-hour analytical sequence, at a minimum, by the injection of a Continuing Calibration Verification standard (CCV) at a midpoint concentration. The 12-hour analytical sequence begins with the injection of a calibration verification standard (ICV or CCV) and ends after the completion of the analysis of the last sample or standard injected within 12 hours of the beginning of the sequence. Additionally, certified laboratories performing this method must ensure that a CCV standard is also injected at the end of the analytical batch (see definition in the 2016 TNI standard per reference 6). The CCV must be evaluated prior to the analysis of any additional samples or acceptance of sample results within the sequence.

10.9.2.1 Evaluation of the CCV

The Percent Difference for the CCV result must be ≤25%. Calculate the Percent Difference for the CCV result as follows.

$$\text{Percent Difference} = \frac{(\text{R1} - \text{R2})}{\text{R1}} \times 100$$

Where:

R1 = Average CF from the initial calibration curve
R2 = Calibration Factor from the CCV

10.9.2.2 Acceptance of Continuing Calibration

If the Percent Difference for the CCV is greater than 25% and the CCV failure is determined to have only impacted the CCV result, immediately analyze another CCV and evaluate the result. If the second CCV meets the verification acceptance criterion, sample results bracketed by the second CCV and the beginning CCV results for the analytical sequence can be accepted, and the analysis of additional samples for the next analytical sequence may proceed. Document the cause of the CCV failure and reanalysis. If the second CCV result fails the acceptance criterion, or a second CCV cannot be analyzed, perform any necessary corrective actions, analyze a new calibration curve, and evaluate the initial calibration for acceptance prior to analyzing any samples (10.9.1.7). Qualify the results of samples not bracketed by acceptable CCV analyses.

10.9.3 Retention Time Window for Calibrations

The retention times for the OTP and C_{39} surrogates, and the C_8 and C_{40} alkanes for all calibration verifications must be within the established range (see 10.10.2 below). If they are out of acceptance range, a new initial calibration must be prepared and verified before samples are analyzed (see 10.9.1).

10.9.4 Normally, calibration verifications should demonstrate acceptable recoveries for verification standards. Corrective actions for failed verifications should ensure that the GC system is properly maintained, including injector components and chromatography columns. Since the heavier PHS components elute later, their absence or low recoveries can also mean that maintenance or cleaning is required before proceeding with a new initial calibration.

10.10 Retention Time Window Definition

The following subsections describe one approach that may be used to establish retention time (RT) windows. Other approaches may be employed, provided the laboratory can demonstrate performance appropriate for this FL-PRO method.

10.10.1 Before establishing windows, be certain that the GC system is within optimum operating conditions. Make three injections of the PHS mix throughout the course of a 72-hour period. Serial injections over less than a 72-hour period result in RT windows that are too tight.

10.10.2 Calculate the standard deviation of the absolute RTs for the two standards, C_8, and C_{40}.

10.10.2.1 The RT window for individual peaks is defined as a plus or minus three times the standard deviation of the absolute RT for each component, or 0.03 minutes, whichever is greater.

10.10.2.2 In those cases where the standard deviation for an individual component peak is zero, either collect data from additional injections of standards, or use a default standard deviation of 0.01 minutes.

10.10.2.3 Calculate the standard deviation of the absolute RTs for the OTP and C_{39} surrogate standards.

10.10.2.4 Determine the center of the RT window for each of the alkane and surrogate standards above from the RT for each compound in the calibration verification standard analyzed at the beginning of the analytical sequence.

10.10.2.5 Calculate retention time windows for the above standards on each GC column and whenever a new GC column is installed. The data must be retained by the laboratory.

10.11 Gas Chromatograph Analysis

Samples are analyzed by GC/FID per conditions and GC system performance criteria in section 10.8.

10.11.1 Suggested injection volumes are 1 - 2 μL. Other injection volumes may be used, including those employing large volume injector configurations.

10.11.2 If an initial calibration (10.9.1) has already been performed, verify the calibration by analysis and evaluation of a mid-point CCV (10.9.2) at the beginning (and end) of each analytical batch or sequence, not to exceed 12 hours between CCVs. The initial verification

using the ICV standard (10.9.1.7) may be used for the start of the first sequence. Certified laboratories must analyze a CCV standard at the end of the analytical batch.

10.11.3 Evaluate the initial or continuing calibration verification responses per 10.9.1.7(a) and 10.9.2.1. If verification responses do not meet the performance criteria specified in the above sections, the instrument must be recalibrated (10.9.1) and all samples which were injected after the failed standard must be qualified or reanalyzed.

10.11.4 Analyze a methylene chloride solvent blank in every analytical sequence to determine the area generated on normal baseline bleed under the conditions prevailing in the 12-hour period. The baseline area is determined by continuous integration of all responses under the same conditions as the standards and samples (i.e., forced baseline and predetermined RT window, per 10.9.3 and 10.10). Do not subtract the baseline. The calculated value (PRO concentration) of the solvent blank must be less than the method detection limit determined by the laboratory for routine analyses of samples of the associated matrix.

10.11.4.1 Methylene chloride solvent blanks should also be analyzed to monitor for carryover in the analytical sequence after analyzing samples suspected of being highly concentrated, or after analyzing CCV standards.

10.11.4.2 If the solvent blank analysis shows contamination, the column must be baked out and subsequent solvent blanks analyzed until the system is shown to be free from contaminants.

10.11.5 The method blank is analyzed in the same manner as the samples. The PRO concentration analyzed in method blanks must be less than the method detection limit determined by the laboratory for routine analyses of samples of the associated matrix. See 11.2.1.

10.11.6 Where practical, samples with unusually high concentrations of analytes should be followed by injection of method blanks or solvent blanks. If target compounds present in an unusually highly concentrated sample are also found to be present in subsequent samples, the analyst must demonstrate that the results for compounds detected in subsequent samples are not affected by carryover contamination.

10.11.7 If the extract concentration of any sample, QC sample or the concentration of a verification standard exceeds the concentration of the highest standard used to generate the initial calibration curve, the sample or standard must be diluted and reanalyzed.

10.11.8 Qualitative Identification by Pattern Recognition (Optional)

The laboratory may qualitatively identify the petroleum products analyzed in samples using FL-PRO.

10.11.8.1 The experience of the analyst weighs heavily in the interpretation of the chromatogram when reporting data by direct comparison of sample chromatograms to retention times and peak patterns of pattern recognition standards.

10.11.8.2 Because of weathering, the patterns may not be a perfect match, however, the characteristics of a petroleum hydrocarbon pattern must be present.

10.11.8.3 Environmental samples may contain more than one type of product, and loss of light end components may mean the product has been in the subsurface a longer period of time.

10.11.8.4 References 1,2,3, and 13 contain some background information on hydrocarbon pattern recognition.

10.11.8.5 Qualitative information may be reported on the final laboratory report. This information should include an alkane range. If possible, product identification should be included and may be based on the laboratory library of chromatograms of commercial petroleum products (e.g., diesel, motor oil, etc.).

10.12 Calculations

Integrate the area for **all** peaks eluting from *n*-octane (C_8) through *n*-tetracontane (C_{40}), using a baseline drawn from a baseline point prior to the peak for *n*-octane to a point past the *n*-tetracontane peak, where the baseline returns to normal (see 10.9.1.4). All area including the "hump-a-gram" and surrogate standards must be included. **Do not** integrate valley to valley for individual peaks when calculating total PRO (or PHS) concentration. Refer to EPA SW-846 method 8000D, section 11.10 (Reference 15) for additional guidance for applicable calculation options for the calibration technique used for sample analyses (10.9).

10.12.1 The responses for surrogate standards must be integrated separately using valley to valley integration, with the concentration of the surrogates calculated from the surrogate single-point response or surrogate calibration curve, and subtracted from the PRO (PHS) concentration calculated from the baseline to baseline integration.

10.12.2 The following formulas for calculating final PRO sample concentrations (or calibration verification standards as PHS) are based on the external standard technique, using either the calibration factor (average response factor) or linear regression calibration models.

10.12.2.1 Calculation of Sample Results Using a Calibration Factor

Sample or CV Standard Concentration:

$$Cs = \frac{(Ax)(Ve)(D)}{(CF)(Inj)(Vs)}$$

Where:

Cs = Concentration of PRO or PHS (mg/L or mg/kg)

Ax = Response for the PRO or PHS in the sample (baseline to baseline integration), units in area

Ve = Final volume of sample extract (mL); For CV standards (PHS), the default volume is the same as sample extract final volume.

D = Dilution factor (dimensionless), if dilution was performed on the sample prior to analysis. If no dilution was made, D = 1

CF = Average Calibration Factor from **initial** calibration (10.9.1.5)

Inj = Volume of injected sample (µL)

Vs = Volume of sample extracted in mL (10.2 or 10.3), or weight of sample extracted in g (10.4 or 10.5); For CV standards (PHS), the default volume or weight is the same as sample extracted volume or weight

10.12.2.2 Calculation of Sample Results Using Linear Regression

(a) Linear Equation

$$Ce = A + BAx$$

Where:

Ce = Concentration of PRO or PHS in extract (µg/mL)

Ax = Response for the PRO or PHS in the sample (baseline to baseline integration), units in area

A = y-intercept value

B = Slope

(b) Sample or CV Standard Concentration

$$Cs = \frac{(Ce)(Ist)(Ve)(D)}{(Inj)(Vs)}$$

Where:

Cs = Concentration PRO or PHS (mg/L or mg/kg)

Ce = Concentration in extract determined from linear regression (µg/mL)

Ist = Amount of each standard injected (µL) - must be constant for all standards

Ve = Final volume of sample extract (mL); for CV standards (PHS), the default volume is the same as sample extract final volume.

D = Dilution factor (dimensionless), if dilution was performed on the sample prior to analysis. If no dilution was made, D = 1

Inj = Volume of injected sample (µL)

Vs = Volume of sample extracted in mL (10.2 or 10.3) or weight of sample in g (10.4 or 10.5); for CV standards (PHS), the default volume or weight is the same as sample extracted volume or weight.

10.12.3 Calculate the dry weight corrected analyzed concentration of solid samples using the percent moisture determination from 10.4.2.

$$\text{mg/(dry kg)}\ \ \text{PH} = \frac{\text{Cs}}{1 - (\%\ \text{moisture}/100)}$$

Where:

Cs = Concentration of PRO (mg/L or mg/kg) calculated in 10.12.2.1 or 10.12.2.2

11. Quality Control

Essential, ongoing quality control (QC) procedures and evaluation criteria must be implemented for all FL-PRO analyses. See the general procedures for QC for chromatographic analyses in EPA SW-846 method 8000D (Reference 15), which may be used as additional guidance for performing the required QC measures described in this section. Certified laboratories must follow all required QC procedures and associated documentation requirements per relevant accreditation standards pursuant to the laboratory's current certification by the Florida Department of Health ELCP (NELAC or TNI, as applicable; Reference 6). See further discussion below concerning requirements for certified labs.

11.1 Before first use of the FL-PRO method, and whenever instrument maintenance or repair is likely to affect performance; or, whenever an approved modification to the FL-PRO method is implemented (section 12), the laboratory must demonstrate initial capability to analyze samples per all QC requirements in the FL-PRO method prior to analysis of client samples from a specified matrix.

11.1.1 Certified environmental laboratories must ensure that all Demonstrations of Capability are met according to current accreditation standards specified by the Florida Department of Health Environmental Laboratory Certification Program (ELCP), as applicable to each analyst performing the method within the laboratory.

11.1.2 Ongoing QC measures performed by the laboratory must include the analysis of QC samples. The laboratory performance must meet the requirements for acceptance of QC data described in section 11.2.

11.1.3 The laboratory Method Detection Limit (MDL) and Practical Quantitation Limit (PQL) or Limit of Quantitation (LOQ) must be determined and verified according to all requirements specified by DEP rules (if applicable) and the current accreditation standards specified by the ELCP (for certified environmental laboratories). Determine the MDL and PQL for each matrix for which the laboratory holds certification for the FL-PRO method. See 2.2.

11.1.4 The laboratory must establish the ability to generate acceptable accuracy and precision and monitor laboratory contamination per section 11.2.

11.1.5 The laboratory's demonstration of acceptable QC data must include the calculation of average recovery and the standard deviation of the recovery; and, relative percent difference (RPD) or percent relative standard deviation (%RSD) for analytical duplicates or replicates, as outlined in Method 8000D, Section 9.4. See 11.2.2.

11.1.6 See section 9.6 in method 8000D for guidance in evaluating QC results for accuracy and precision.

11.1.7 Evaluate the results of method blank analyses per section 11.2.1.

11.2 A method blank, matrix spike (MS), matrix spike duplicate (MSD) or sample duplicate (SD), and Laboratory Control Sample (LCS) must be processed with each preparation (extraction) batch of 20 samples or less. The method blank and LCS must be prepared, analyzed and evaluated per the current accreditation standards adopted by the ELCP certification rules. The use and evaluation of MS/MSD, SD and surrogates should likewise meet current accreditation requirements, and the laboratory must meet the corresponding requirements in this FL-PRO method. In lieu of other criteria justified by the laboratory, DEP recommends spiking the LCS at 1-4X the claimed laboratory PQL, and spiking the surrogate standards in the range of 50 – 200 mg/L or in the middle of the calibration range. Other surrogate concentrations may be used for spiking samples for MDL studies (2.2). See EPA SW-846 method 8000D for additional guidance about LCS and surrogate spikes.

11.2.1 The calculated PRO value of the method blank must be <MDL. Method blank values at or above the current laboratory MDL must be evaluated against the associated samples in the preparation batch. Any affected sample results must be reported with the "V" data qualifier code per the requirements for use of the code in Table 1 (Data Qualifier Codes), at 62-160.700, Florida Administrative Code (F.A.C.).

11.2.2 The calculation of average recovery and the standard deviation of the recovery for the LCS, surrogate spike and MS/MSD must be followed as outlined in Method 8000D, Section 9.0 (Quality Control). If the matrix spike(s) are not within the established laboratory control limits for the specific type of QC sample, sample preparation procedure, and matrix, the results of the LCS and any other analyzed QC check samples must be evaluated for method control.

11.2.2.1 If the results of the LCS, surrogate or QC check sample are not within specified limits, determine the potential cause of the QC failure and repeat the preparation and analysis of the affected sample(s) in the preparation batch, or qualify the sample results as estimated values in the analytical report, using the "J" qualifier code and explanation, per Table 1 in rule 62-160.700, F.A.C.

11.2.2.2 If the results of the LCS and QC check samples are within specified limits, a problem with the surrogate or MS/MSD samples may be assumed to be attributed to matrix interferences, and only the parent sample result must be reported as qualified with a "J" data qualifier code and explanation of the QC failure, per Table 1 at 62-160.700, F.A.C.

11.2.2.3 See Tables 4 and 5 (Section 15) for example control limits for accuracy and precision, which may be used as guidance for method and matrix-specific control of recovery and RPD (or %RSD) until sufficient QC data is collected from the analyses of QC samples. When sufficient QC results are available, the laboratory must generate in-house control limits based on its implementation of the FL-PRO method specific to its standard operating procedures (SOP), including any approved method modifications. See 11.2.2.5.

11.2.2.4 Compare in-house laboratory limits with the corresponding limits in Tables 4 and 5. If the laboratory limits are less stringent than those listed in the tables, evaluate calculations and QC procedures for possible errors. See section 13.

11.2.2.5 Surrogate spike recovery for each analyzed field sample, QC sample and calibration standard must be calculated and evaluated per established laboratory control limits for the surrogate recovery. If recoveries are outside established limits, verify calculations, dilutions, standard solutions and instrument performance.

(a) **Unlike the calculation of petroleum hydrocarbons (PRO), the surrogate spike response must be based on a valley-valley integration of the peak response.**

(b) High recoveries may be due to a coeluting matrix interference or the presence of high molecular weight contaminants; examine the sample chromatogram.

(c) High recoveries may also be due to memory effects caused by poor sample volatility, backflash or carryover. Check instrument conditions, injection volume and injector temperature.

(d) Low recoveries may be due to adsorption by the sample matrix. For the C_{39} surrogate, low recoveries can indicate solubility problems with the spiking standard solutions or spiked samples, or rapid degradation of recovery with successive injections. Low recoveries of surrogate mixes may also indicate that injector or GC column maintenance is required. Instrument calibration verified with second-source verification standards can assist in ensuring that low surrogate recoveries are not related to a calibration issue.

(e) If the surrogate recovery is outside of established limits, the associated sample results must be flagged with the "J" qualifier code and an explanation provided, per Table 1 at 62-160.700, F.A.C.

11.2.2.6 The LCS and surrogate recovery control limits must be used to evaluate recoveries for all demonstrations of capability performed per section 11.1.1.

11.2.2.7 The accuracy and precision acceptance limits for all QC samples should be recalculated and updated at least annually or more frequently as determined by the laboratory, according to the internal laboratory SOP for QC procedures, or requirements in the laboratory quality manual.

12. Method Modifications

The procedures in the FL-PRO method define and determine how petroleum hydrocarbon components in a sample will be analyzed and quantified. As such, data generated from FL-PRO analyses represent a quantitative characterization of "method-defined" analytes. Modifications to the FL-PRO method are approved by DEP if the qualitative and quantitative characterization of the sample FL-PRO data produced by the modified method are equivalent to data generated using the procedures in this FL-PRO method, and all quality control requirements of this FL-PRO method can be achieved using the modifications. DEP will not approve any modifications that do not allow the use of the modified FL-PRO method to achieve the Department's data quality objectives for any use of FL-PRO data. For additional guidance describing the types of typical modifications allowed, see the discussion at 40 CFR, section 136.6 (Reference 19), and in Chapter Two, section 2.1, in SW-846 (Reference 15). Allowed modifications to this FL-PRO method are dependent on the following conditions:

12.1 Ensure that laboratory modifications to FL-PRO meet applicable requirements in 62-160.220, 62-160.400, and 62-160.330, F.A.C. The requirements in those rules govern whether DEP must review the laboratory method validation data for approval of the modifications prior to their use; or, the modifications are pre-approved as allowed by this FL-PRO method and summarized below.

12.2 The laboratory must demonstrate that any modification to FL-PRO produces performance data that are equivalent or superior to the performance data of this published FL-PRO method, when analyzing samples to meet DEP data quality objectives for a specified use of the data. See the cited Chapter 62-160 rules for further information (section 12.1 above). This demonstration must be performed as part of the initial demonstration of capability, with documentation of the demonstration maintained by the laboratory, and available for inspection upon request from DEP. The documentation must include the performance data and supporting records, such as extraction logs, calibration and spiking standard logs, calibration and QC data, and chromatograms, as well as a detailed description of the procedural steps as performed, typically written as an internal standard operating procedure (SOP).

12.3 Method modifications that alter any sample collection, preservation, or holding time procedures or requirements for the FL-PRO samples must be approved by DEP according to section 12.1. Any changes that would alter the defined chemistry (e.g., changing the extracted hydrocarbon range or detector type) are prohibited.

12.4 Examples of acceptable method modifications include changes in sample collection volume, changes in solvent volumes, changes in solvents used to prepare standards, use of different

sample extraction equipment, use of alternative and/or automated sample extract concentrators, changes in the final sample extract volume, use of different GC columns, changes in temperature programs in GC columns and injectors, and using different concentrations of calibration standards, spiking standards and surrogate standards.

12.4.1 Changes to sample preparation procedures may include modifications to liquid-liquid extraction procedures, including changes based on EPA SW-846 method 3511 (Reference 15), that reduce sample extracted volume, extraction solvent volume, and/or the final volume of extract.

12.4.2 Laboratories utilizing micro-extraction methods frequently use large volume injection (LVI) techniques to achieve the required MDLs. Whenever “solvent vent” protocols are employed for LVI, ensure that the recoveries for the low-end hydrocarbons (especially C_8) are not adversely affected.

12.4.3 Changes to non-aqueous sample preparation may include extractions using microwave vessels, pressurized vessels, heating blocks, and automated Soxhlet extractors; and, less common preparations such as supercritical fluid extraction and microscale extraction; based on EPA SW-846 methods 3541, 3545A, 3546, 3560, and 3570 (Reference 15).

12.4.4 In general, the following EPA SW-846 methods may be used as guidance for modifications to analytical procedures in this FL-PRO method (8000D, 8015D), or modifications to the sample extraction procedures (3500, 3510C, 3511, 3520C, 3535A, 3540C, 3541A, 3545, 3546, 3550C, 3560, 3570, 3580, 3600, 3630C). See Reference 15.

13. Method Performance

The method performance data presented in the tables in Section 15 below should be used as guidance in evaluating internal performance data generated by the laboratory, until enough internal data has been collected to develop in-house QC limits. The tables are specific to the applicable sample preparation methods and sample matrices indicated. The acceptance limits in Tables 4 and 5 are the minimum performance requirements for recovery and precision when using this method.

13.1 Each laboratory must develop internal quality control limits for precision and accuracy, utilizing data collected from the ongoing analyses of laboratory control samples, surrogate spikes, matrix spikes and laboratory duplicate samples (or replicates), per section 11 (Quality Control). All laboratory QC data must be generated using the routine laboratory SOP, including all modifications made to the FL-PRO method.

13.2 Compare internally generated QC limits with tables 4 and 5 in Section 15 for each matrix and QC sample analyzed by the laboratory.

13.2.1 If the calculated laboratory limits are not as stringent as the limits in the applicable table (i.e., within the ranges indicated in the tables), evaluate or recalculate the internal QC limits to check for errors in the limit calculations, precision and recovery calculations for individual QC sample results, calculations for spiking standard concentrations or errors in laboratory procedures, and perform necessary corrective actions before proceeding with additional analyses.

13.2.2 Some environmental sample matrices may present analytical interferences that result in poor recoveries for matrix spikes and surrogate spikes, resulting in qualified data.

13.2.3 The LCS and surrogate limits in Tables 4 and 5 must be used to evaluate recovery data for Demonstration of Capability studies (11.1.1), until in-house laboratory control limits have been developed for these QC samples.

14. References

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16. Zills, K., M. McDevitt, and J. Parr; "A Reliable Technique for Measuring Petroleum Hydrocarbons in the Environment," presented at the conference on Petroleum Hydrocarbons and Organic Chemicals in Groundwater, NWWA, Houston, Texas, November 1988.

17. Florida Department of Environmental Protection, DEP SOP FS 1000, General Sampling Procedures, in “Standard Operating Procedures for Field Activities”, DEP-SOP-001/01, January 2017.

18. USEPA, Office of Water, “Definition and Procedure for the Determination of the Method Detection Limit”, Revision 2, December, 2016, EPA 821-R-16-006 (same as 40 CFR, Part 136, Appendix B, July 1, 2018).

19. 40 CFR, Part 136.6, Method Modifications and Analytical Requirements, July 1, 2018.

15. Tables

Table 1

FL-PRO
Components in Petroleum Hydrocarbon Standard (PHS)

Octane (C_{8})
Decane (C_{10})
Dodecane (C_{12})
Tetradecane (C_{14})
Hexadecane (C_{16})
Octadecane (C_{18})
Eicosane (C_{20})
Docosane (C_{22})
Tetracosane (C_{24})
Hexacosane (C_{26})
Octacosane (C_{28})
Triacontane (C_{30})
Dotriacontane (C_{32})
Tetratriacontane (C_{34})
Hexatriacontane (C_{36})
Octatriacontane (C_{38})
Tetracontane (C_{40})

Table 2

FL-PRO
Interlaboratory Performance

Method performance data was generated by an interlaboratory study with 13 participating laboratories using this FL-PRO method. The groundwater and soil samples were supplied by an independent vendor and were spiked with a mixture of diesel, fuel oil and a fatty acid interferent.

Matrix	Spiked Conc.	Number of Labs*	Data Points (n)	Mean %Recovery	Standard Dev.
Water	17.7 mg/L	9	25	66.3	20.5
Water	132 mg/L	9	26	75.2	36.3
Soil (Sonication)	35.2 mg/kg	7	16	163	90.5
Soil (Sonication)	1080 mg/kg	8	23	113	46.1
Soil (Soxhlet)	35.2 mg/kg	7	18	173	114
Soil (Soxhlet)	1080 mg/kg	8	21	98.5	46.5

* Data acceptability was dependent upon the use of the correct integration techniques and/or the Grubbs test for outliers.

Table 3

FL-PRO
Petroleum Product
Single-Laboratory Performance

In a single laboratory study, known concentrations of diesel and motor oil standards were injected and the responses were calculated by this FL-PRO method

% Recovery Product	% Recovery Water	% Recovery Solid Matrix
Diesel	99%	106%
10W40 Motor Oil	78%	101%

Note for tables 4 and 5:

Spike recovery limits were calculated based on ten years of data collected at the Florida DEP Chemistry Laboratory between January 1, 2006 and December 31, 2015, using this FL-PRO method (1-liter separatory funnel water extraction, soil sonication and waste dilution techniques). The acceptance criteria for Laboratory Control Samples and surrogates must be used to assess a laboratory's Demonstration of Capability study results for the initial and ongoing use of the FL-PRO method. The LCS limits, along with limits for matrix spikes (PHS and surrogate analytes), may be used on a temporary basis, until the laboratory acquires enough statistical data to calculate internal recovery limits. See section 11 (Quality Control) and Section 13 (Method Performance).

Table 4

FL-PRO
Single-Laboratory Method Acceptance Criteria
Recovery and Precision

Spiked Mixture	Matrix	% Recovery*			Precision (%RSD)		
		Water 1 Liter Sep. Funnel	Soil 25 g (Sonication)	Waste Dilution	Water	Soil	Waste
PHS	LABORATORY CONTROL SAMPLES	66-119	65-119	70-114	0-20	0-25	0-25
PHS	MATRIX SPIKES	65-123	39-181	14-197	0-20	0-25	0-25

*Recoveries based on average spike levels for water (2 mg/L), soils (80 mg/kg) and wastes (2000 mg/kg). Final extract volume was 2 mL.

Table 5

FL-PRO
Single-Laboratory Method Acceptance Criteria
Surrogate Recovery

Surrogate	% Recovery* Water (1 Liter) Sep. Funnel	% Recovery* Soil (25g) Sonication	% Recovery* Waste (Dilution)
OTP	66-139	66-136	61-138
C_{39}	40-129	36-132	27-141

*Recoveries based on average spike levels for water (0.2 mg/L), soils (8 mg/kg) and wastes (200 mg/kg). Final extract volume was 2 mL.