Title: Determination of Table 6 Compounds by LC/MS/MS
Chemours Fluoroproducts

Approvals (Signature/Date):

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1. SCOPE AND APPLICATION

1.1. This procedure describes the analysis of water samples for the following compounds using liquid chromatography / tandem mass spectrometry (LC/MS/MS).

Table 6 Compound Name	Abbreviation	CAS #
PEPA	PEPA	267239-61-2
PMPA	PMPA	13140-29-9
DFSA	DFSA	422-67-3
MMF	MMF	1514-85-8
MTP	MTP	93449-21-9
PPF Acid	PPF Acid	422-64-0

1.2. The working range of the method is listed below. The linear range can be extended by diluting the extracts.

Matrix	Nominal Sample Size	Reporting Limit	Working Range
Water	250 mL	2 ng/L – 4 ng/L	2 ng/L – 2000 ng/L

2. SUMMARY OF METHOD

2.1. A 250 mL aliquot of aqueous sample is loaded onto a [redacted] cartridge containing [redacted]. The PFAS compounds are eluted with [redacted]. The extract is then fortified with 2 mL of [redacted] and concentrated to 1.5 mL using nitrogen in a heated water bath. The samples are brought to 2 mL using [redacted], internal standard is spiked and samples are adjusted to a final volume of 10 mL using [redacted].

2.2. A fixed volume, such as 20 µL, is injected on the HPLC equipped with a C18 column interfaced to a tandem mass spectrometer (LC/MS/MS). The mass spectrometer detector is operated in the electrospray (ESI) negative ion mode for the analysis of analytes

2.3. An isotope dilution technique is employed with this method for the compounds of interest. The isotope dilution analyte (IDA) consist of a carbon-13 labeled analyte, and is spiked into the samples at the time of extraction. This technique allows for the correction for analytical bias encountered when analyzing more chemically complex environmental samples. The isotopically labeled compound is closely related chemically to the compounds of concern and therefore affected by sample-related interferences to the same extent as the compounds of concern.

2.4. Quantitation by the internal standard method is employed for the IDA analytes/recoveries. Peak response is measured as the area of the peak.

3. DEFINITIONS

3.1. PFAS: Per- and Polyfluorinated Alkyl Substances

3.2. PTFE: Polytetrafluoroethylene (e.g. Teflon®)

3.3. IDA: Isotope Dilution Analyte

3.4. PP: Polypropylene

3.5. PE: Polyethylene

3.6. HDPE: High density polyethylene

3.7. Further definitions of terms used in this SOP may be found in the glossary of the Laboratory Quality Assurance Manual (QAM).

4. INTERFERENCES

4.1. PFAS have been used in a wide variety of manufacturing processes, and laboratory supplies should be considered potentially contaminated until they have been tested and shown to be otherwise. The materials and supplies used during the method validation process have been tested and shown to be clean. These items are listed below in Section 6.

4.2. To avoid contamination of samples, standards are prepared in a ventilation hood in an area separate from where samples are extracted.

4.3. The use of PTFE in the procedure should be avoided or at least thoroughly tested before use. Polypropylene (PP) or polyethylene (PE, HDPE) products may be used in place of PTFE products to minimize PFOA or other related analyte contamination.

4.4. Standards and samples are injected from polypropylene auto sampler vials with polypropylene screw caps once. Multiple injections may be performed on Primers when conditioning the instrument for analysis.

4.5. Teflon-lined screw caps have detected PFAS at low concentrations. Repeated injection from the same Teflon-lined screw cap have detected PFNA at increasing concentration as each repeated injection was performed, therefore, it is best to use polypropylene screw caps.

4.6. Volumetric glassware and syringes are difficult to clean after being used for solutions containing high levels of these analytes. These items should be labeled for use only with similarly concentrated solutions or verified clean prior to re-use. To the extent possible, disposable lab ware is used.

4.7. Matrix interferences may also be caused by contaminants that are co-extracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending on the nature of the sample. Humic and/or fulvic material is co-extracted by this method and high levels can cause enhancement and/or suppression in the electrospray ionization source or low recoveries on the SPE sorbent. Total organic carbon (TOC) is a good indicator of humic content of the sample.

4.8. SPE cartridges can be a source of interferences. The analysis of field and laboratory reagent blanks can provide information regarding to the presence or absence of such interferences. Brands and lots of SPE devices should be tested to ensure that the contamination does not preclude analyte identification and quantitation.

5. SAFETY

Employees must abide by the policies and procedures in the Corporate Safety Manual, Sacramento Supplement to the CSM, and this document. All work must be stopped in the event of a known or potential compromise to the health or safety of an associate. The situation must be reported **immediately** to a supervisor, the EH&S Staff, or a senior manager.

5.1. Specific Safety Concerns

Preliminary toxicity studies indicate that PFAS could have significant toxic effects. In the interest of keeping exposure levels as low as reasonably achievable, PFAS and PFAS samples must be handled in the laboratory as hazardous and toxic chemicals.

5.1.1. Laboratory procedures such as repetitive use of pipets, repetitive transferring of extracts and manipulation of filled separatory funnels and other glassware represent a significant potential for repetitive motion or other ergonomic injuries. Laboratory associates performing these procedures are in the best position to realize when they are at risk for these types of injuries. Whenever a situation is found in which an employee is performing the same repetitive motion, the employee shall immediately bring this to the attention of their supervisor, manager, or the EH&S staff. The task will be analyzed to determine a better means of accomplishing it.

5.1.2. Ensure that the vacuum exhaust hose used during the filtering is securely anchored inside of a fume hood so that vapors are not pumped into the working environment.

5.1.3. Exposure to chemicals must be maintained as low as reasonably achievable; therefore all samples must be opened, transferred and prepared in a fume hood. Solvent and waste containers will be kept closed unless transfers are being made.

5.1.4. Eye protection that satisfies ANSI Z87.1 (as per the TestAmerica Corporate Safety Manual), laboratory coat, and nitrile gloves must be worn while handling samples, standards, solvents, and reagents. Disposable gloves that have been contaminated will be removed and discarded; other gloves will be cleaned immediately.

5.2. Primary Materials Used

The following is a list of the materials used in this method, which have a serious or significant hazard rating. NOTE: This list does not include all materials used in the method. The table contains a summary of the primary hazards listed in the SDS for each of the materials listed in the table. A complete list of materials used in the method can be found in the reagents and materials section. Employees must review the information in the SDS for each material before using it for the first time or when there are major changes to the SDS.

Material[(1)]	Hazards	Exposure Limit[(2)]	Signs and Symptoms of Exposure
[redacted] (2-3-0)	Flammable Poison Irritant	200 ppm (TWA) 250 ppm (STEL)	A slight irritant to the mucous membranes. Toxic effects exerted upon nervous system, particularly the optic nerve. Symptoms of overexposure may include headache, drowsiness and dizziness. Methyl alcohol is a defatting agent and may cause skin to become dry and cracked. Skin absorption can occur; symptoms may parallel inhalation exposure. Irritant to the eyes.
[redacted] (3-1-0)	Corrosive Poison	50 ppm-TWA	Severe irritant. Effects from inhalation of dust or mist vary from mild irritation to serious damage to the upper respiratory tract. Symptoms may include sneezing, sore throat, or runny nose. Contact with skin can cause irritation or severe burns and scarring with greater exposures. Causes irritation of eyes, and with greater exposures it can cause burns that may result in permanent damage, including blindness. Brief exposure to 5000 PPM can be fatal.
(1) Always add acid to water to prevent violent reactions. (2) Exposure limit refers to the OSHA regulatory exposure limit.			

6. EQUIPMENT AND SUPPLIES

6.1. 250 mL HDPE bottles with HDPE screw caps.

6.2. Analytical balance capable of accurately weighing to the nearest 0.0001 g, and checked for accuracy each day it is used in accordance with WS-QA-0041.

6.3. Auto-pipets capable of accurately dispensing volumes of 2.5 mL, 2.0 mL, and 0.5 mL, and other equipment used to prepare standards and reagents.

6.4. Extract concentrator or nitrogen manifold with water bath heating to 55°C.

6.5. Pipettes, auto-pipets, and other equipment used to prepare standards and reagents.

6.6. Solid phase extraction (SPE) system.

6.6.1. SPE Cartridges – [redacted] cartridges containing [redacted] (Phenomenex Product Lot: [redacted]), or equivalent.

6.6.2. Vacuum extraction manifold – Supelco Visiprep, or equivalent. A manual vacuum manifold with column adapters, and column reservoirs for cartridge extraction.

6.7. Test tubes, 15 mL, polypropylene, with polypropylene screw caps.

6.8. Eppendorf 1000 µL epTIPS, part no. 022491954 or equivalent.

6.9. 1000 µL Pipette: Eppendorf Research Plus

6.10. Miscellaneous laboratory apparatus (beakers, test tubes, volumetric flasks, pipettes, etc.). These should be disposable where possible, or marked and segregated for high-level versus low-level use.

6.11. Liquid Chromatography/Tandem Mass Spectrometer (LC/MS/MS) – described below, or equivalent, may be used for this method. The HPLC is equipped with a refrigerated auto sampler, an injection valve, and a pump capable of variable flow rate. The use of a column heater is required to maintain a stable temperature throughout the analytical run. Data is processed using Chrom Peak Review, version 2.1 or equivalent.

This system consists of a SCIEX HPLC interfaced with a SCIEX 5500 Triple Quad MS. The instrument control and data acquisition software is SCIEX Analyst, version 1.6.3 or equivalent.

6.11.1. SCIEX HPLC equipped with 3 LC-20AD pumps, one DGU-20 degassing unit, and a rack changer or equivalent.

6.11.2. Phenomenex [redacted], Part No. [redacted], or

equivalent.

6.11.3. PFAS Isolator column, Phenomenex [redacted], part no. [redacted] or equivalent. This is plumbed between the UPLC pumps and auto sampler valve to minimize PFAS background from the UPLC solvent lines and filters.

6.11.4. KrudKatcher ULTRA HPLC In-Line Filter, Part No. AFO-8497. This is plumbed in front of the Phenomenex [redacted] column.

6.12. Preventive and routine maintenance is described in the table below

HPLC/MS/MS Preventative Maintenance	
As Needed: Change pump seals. Change in-line filters in auto sampler (HPLC). Check/replace in-line frit if excessive pressure or poor performance. Replace column if no change following in-line frit change. Clean corona needle. Replace sample inlet tube in APCI (10.1 cm). Replace fused silica tube in ESI interface. Clean lenses. Clean skimmer. Ballast rough pump 30 minutes. Create all eluents in Reagent module, label eluent containers with TALS label and place 2nd label into maintenance log when put into use.	**Daily (When in use)** Check solvent reservoirs for sufficient level of solvent. Verify that pump is primed, operating pulse free. Check needle wash reservoir for sufficient solvent. Verify capillary heater temperature functioning. Verify vaporizer heater temperature. Verify rough pump oil levels. Verify turbo-pump functioning. Verify nitrogen pressure for auxiliary and sheath gasses. Verify that corona and multiplier are functioning.
Semi-Annually Replace rough-pump oil (4-6 months). Replace oil mist and odor elements. Replace activated alumina filter if applicable	**Annually** Vacuum system components including fans and fan covers. Clean/replace fan filters, if applicable.

7. REAGENTS AND STANDARDS

7.1. Reagent grade chemicals shall be used in all tests whenever available. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the Committee on the Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided it is first ascertained

that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination.

7.2. [redacted]. This solution is volatile and should be replaced every 7 days or sooner.

7.3. [redacted]

7.4. [redacted].

7.5. Water, Nanopure or Millipore, must be free of interference and target analytes

7.6. Standards

7.6.1. Most analytes were provided by Chemours, as high purity solids (96% or greater) or as certified solutions. The solid stock material is stored at room temperature or as specified by Chemours.

7.6.2. Individual and mixed stock solutions are available from reputable vendors such as Wellington Laboratories.

7.6.3. While standards commercially purchased are supplied in glass ampoules, all subsequent transfers or dilutions performed by the analyst must be prepared and stored in polypropylene or HDPE containers.

7.7. Calibration Standards

7.7.1. The stock solution is prepared by diluting the appropriate amounts of the stock analytes to create a solution at 1 ppm in [redacted]. The stock solution should be stored in a refrigerator when not in use. An aliquot is diluted 20X and 200X with [redacted] to produce a 50 ppb and 5 ppb standard. These standards are then used for spiking and to prepare dilutions for the calibration curve. [redacted].

7.7.2. Table 6 IDA intermediate (IM) solution – Prepare a Table 6 IDA IM solution by adding the appropriate amount of the stock solution in [redacted]. The resultant mixture is 0.5 µg/mL. The solution is stored in a polypropylene bottle at 0 – 6°C and is valid for 6 months.

7.7.3. Table 6 IDA working solution – Dilute 10 mL of the 0.5 µg/mL IM solution to a final volume of 200 mL in [redacted] for a 20X dilution. The resultant

concentration is 0.025 µg/mL in [redacted]. The solution is stored in a polypropylene bottle at 0 – 6°C and is valid for 6 months.

Composition of Table 6 IDA working solution			
Table 6 IDA Analyte	**IM Conc. (µg/mL)**	**Aliquot (mL) to 200mL final volume**	**Table 6 IDA working solution Conc. (µg/mL)**
13C4-PFBA	50	10	0.25

7.7.4. Table 6 IS solution – A Table 6 Internal Standard Solution is prepared by diluting the appropriate amount of stock solution in 200 mL of [redacted]. Stored in a polypropylene bottle at 0 – 6°C, and is valid for 6 months

Initial Calibration (ICAL) Levels (ng/mL)										
Table 6 Compounds	**CS-1**	**CS-2**	**CS-3**	**CS-4**	**CS-5**	**CS-6**	**CS-7**	**CS-8**	**CS-9**	**CS-10**
PEPA	0.025	0.05	0.25	0.5	1	2.5	5	10	20	50
PMPA	0.025	0.05	0.25	0.5	1	2.5	5	10	20	50
DFSA	0.025	0.05	0.25	0.5	1	2.5	5	10	20	50
MMF	0.025	0.05	0.25	0.5	1	2.5	5	10	20	50
MTP	0.025	0.05	0.25	0.5	1	2.5	5	10	20	50
PPF Acid	0.025	0.05	0.25	0.5	1	2.5	5	10	20	50
IDA										
13C4-PFBA	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25
IS										
13C2-PFOA	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25

Note: Not all calibration points are used for all analytes.

7.8. Initial Calibration Verification Standard (ICV)

For these analytes, a second analyst will prepare a second source standard from the same source as the ICAL to produce an ICV. The recommended concentration of the ICV standard should be in the mid-range of the calibration curve. The concentration may be adjusted if the initial calibration levels are changed or altered.

7.9. LCS/Matrix Spike Solution, 5 ng/mL

The spike solution is prepared by using the solution described in Section 7.7.1. This solution contains each analyte at a concentration of 5 ng/mL in [redacted].

8. SAMPLE COLLECTION, PRESERVATION, AND STORAGE

8.1. Water samples are collected in pre-cleaned 250 mL HDPE containers. Other containers may also be suitable. Samples are chilled to 0 - 6°C for shipment to the laboratory.

8.2. Samples are logged in following normal laboratory procedures and are stored under refrigeration at 0 - 6°C. Water samples should be analyzed within 28 days of collection.

9. QUALITY CONTROL

9.1. Initial Demonstration of Capability

The initial demonstration and method detection limit (MDL) studies described in Section 13 must be acceptable before analysis of samples may begin.

9.2. Batches are defined at the sample preparation step. Batches should be kept together through the whole analytical process as far as possible, but it is not mandatory to analyze prepared extracts on the same instrument or in the same sequence. Refer to the QC program document (WS-PQA-003) for further details of the batch definition.

9.2.1. The quality control batch is a set of up to 20 samples of the same matrix processed using the same procedure and reagents within the same time period. The quality control batch must contain a matrix spike/matrix spike duplicate (MS/MSD), a laboratory control sample (LCS) and a method blank. Laboratory generated QC samples (Blank, LCS, MS/MSD) do not count toward the maximum 20 samples in a batch. Field QC samples are included in the batch count. In some cases, at client request, the MS/MSD may be replaced with a matrix spike and sample duplicate. If insufficient sample is available for an MS/MSD an LCSD may be substituted if required by the program or client. In the event that multiple MS/MSD are run with a batch due to client requirements, the additional MS/MSD do not count toward the maximum 20 samples in a batch.

9.3. One method blank (MB, laboratory reagent blank) must be extracted with every process batch of similar matrix, not to exceed twenty (20) samples. For aqueous samples, the method blank is an aliquot of laboratory reagent water. The method blank is processed in the same manner, , and at the same time, as the associated samples. Corrective actions must be documented on a Non-Conformance memo and implemented when target analytes are detected in the method blank above the reporting limit or when IDA recoveries are outside of the control limits. Re-extraction of the blank, other batch QC, and the affected samples are required when the method blank is deemed unacceptable. See policy WS-PQA-003 for specific acceptance criteria.

9.3.1. If the MB produces a peak within the retention time window of any of the analytes determine the source of the contamination and eliminate the interference before processing samples.

9.3.2. The method blank must not contain any analyte at or above the reporting limit, or at or above 10% of the measured concentration of that analyte in the associated samples, whichever is higher.

9.3.3. If there is no target analyte greater than the RL in the samples associated with an unacceptable method blank, the data may be reported with qualifiers. Such action should be taken in consultation with the client

9.3.4. Re-extraction and re-analysis of samples associated with an unacceptable method blank is required when reportable concentrations are determined in the samples.

9.3.5. Refer to WS-PQA-003 for further details of the corrective actions.

9.4. A laboratory control sample (LCS) must be extracted with every process batch of similar matrix, not to exceed twenty (20) samples. The LCS is an aliquot of laboratory matrix (e.g. water for aqueous samples and Ottawa sand for solids) spiked with analytes of known identity and concentration. The LCS must be processed in the same manner and at the same time as the associated samples. Corrective actions must be documented on a Non-Conformance memo, then implemented when recoveries of any spiked analyte is outside of the control limits. Re-extraction of the blank, other batch QC, and all associated samples are required if the LCS is deemed unacceptable. See WS-PQA-0003 for specific acceptance criteria. The control limits for the LCS are stored in TALS. A matrix spike/matrix spike duplicate (MS/MSD or MS/SD) pair must be extracted with every process batch of similar matrix, not to exceed twenty (20) samples. An MS/MSD pair is aliquots of a selected field sample spiked with analytes of known identity and concentration. The MS/MSD pair must be processed in the same manner and at the same time as the associated samples. Spiked analytes with recoveries or precision outside of the control limits must be within the control limits in the LCS. Corrective actions must be documented on a nonconformance memo, and then implemented when recoveries of any spiked analyte are outside of the control limits provided by TALS or by the client.

9.5. A duplicate control sample (LCSD or DCS) may be added when insufficient sample volume is provided to process an MS/MSD pair, or is requested by the client. The LCSD is evaluated in the same manner as the LCS. See WS-PQA-003 for specific acceptance criteria.

9.6. Initial calibration verification (ICV) –A second source standard is analyzed with the initial calibration curve. The concentration should be at the mid range of the curve.

Corrective actions for the ICV include:

- Rerun the ICV.
- Remake or acquire a new ICV.
- Evaluate the instrument conditions.
- Evaluate the initial calibration standards.
- Rerun the initial calibration.

9.7. Isotope Dilution Analytes

9.7.1. The IDA solution is added to each field and QC sample at the time of extraction, as described in Section 11. As described in Section 7, this solution consists of an isotopically labeled analyte.

9.7.2. IDA recoveries are flagged if they are outside of the acceptance limits (25–150%). Quantitation by isotope dilution generally precludes any adverse effect on data quality due to IDA recoveries being outside of the acceptance limits as long as the signal-to-nose ratio is greater than 10:1.

9.7.2.1. Evaluate data quality for usability, flag and submit a nonconformance memo for any analytes outside of the recovery criteria, and report if data is deemed not adversely effected.

9.7.2.2. If IDA recovery is > 150%, check for laboratory error and correct if identified. If no laboratory error is identified, proceed as follows:

- If field samples are ND for the associated native target analytes, report the data with appropriate qualifiers and narrative comments.
- If field samples are positive for the associated native target analytes and IDA recovery is < 200%, report the data with appropriate qualifiers and narrative comments.
- If field samples are positive for the associated native target analytes and IDA recovery is > 200%, RI at an appropriate dilution then report both sets of data with appropriate qualifiers and narrative comments.

9.7.2.3. If IDA recovery is < 25% check for laboratory error and correct if identified. If no laboratory error is identified, proceed as follows:

- If field samples are positive for the associated native target analytes and IDA recovery is > 10%, report the data with appropriate qualifiers and narrative comments.
- If field samples are ND for the associated native target analytes and IDA recovery is > 10% evaluate the S/N of the associated native analytes in the most recent RL (CCVL) standard or L1 if an ICAL is ran that day.
- If the S/N multiplied by the IDA recovery is > 10, report the data with appropriate qualifiers and narrative comments. For example, if the CCVL has a S/N = 50 * 0.25 (25%) = 12.5. 12.5 > 10 and therefore the RL is supported.
- If the S/N multiplied by the IDA recovery is < 10, report an elevated RL if project DQOs allow, and then qualify and narrate. Otherwise RX or RI at an appropriate dilution. For example, If the CCVL has S/N = 50 * 0.15 (15%) = 7.5. 7.5 < 10 and therefore the RL must be elevated. If the RL is elevated add a comment into the worksheet to notify the 2nd level reviewer.

9.7.2.4. Re-extraction of samples should be performed if the signal-to-noise for any IDA is less than 10:1 or if the IDA recoveries fall below 10%.

9.7.2.5. Re-extraction may be necessary under other circumstances when data quality has been determined to be adversely affected.

9.8. Internal Standard

9.8.1. The Internal Standard (IS) is added to each field and QC samples prior to analysis. The CCV IS response (peak area) must not deviate by more than 50% from the average response (peak area) of the initial calibration.

9.8.2. Sample IS response (peak area) must be within ± 50% of the response (peak area) in the most recent CCV.

9.8.3. If the IS does not meet criteria, re-analyze the extract. If the IS meets criteria in the second analysis, report that analysis. If the IS does not meet criteria in the second analysis, report the first analysis with narration.

10. CALIBRATION

10.1. For details of the calculations used to generate the regression equations, and how to use the factors generated by these equations, refer to SOP CA-Q-P-003 "Calibration Curves and Selection of Calibration Points".

10.2. Routine instrument operating conditions are listed in the table in Section 11.8.

10.3. Instrument Tuning and Mass Calibration

10.3.1. Mass Calibration is performed by instrument manufacturer service representatives in accordance with the manufacturer's procedures during installation, and annually thereafter.

10.3.2. Instrument tuning is done initially when the method is first developed and thereafter as needed during troubleshooting. Tuning is done by infusing each individual compound into the mobile phase using a tee fitting at a point just before the entrance to the electrospray probe. The responses for the parent and daughter ions for each compound are observed and optimized for sensitivity and resolution. Mass assignments are reviewed and updated as needed. The mass assignments must be within ± 0.5 amu of the values shown in the table in Section 11.8.

10.3.3. Once the optimal mass assignments (within ± 0.5 amu of true) are made immediately following the initial tune, the lowest level standard from the initial calibration curve is assessed to ensure that a signal to noise ratio greater than 10 to 1 (S/N > 10:1) is achieved for each PFAS analyte. The first level standard from the initial calibration curve is used to evaluate the tune stability on an ongoing basis. The instrument mass windows are set initially at ± 0.5 amu of the true value; therefore, continued detection of the analyte transition with S/N > 10:1 serves as verification that the assigned mass remains within ± 0.5 amu of the true value.

10.4. A new calibration curve must be generated after major changes to the system or when the continuing calibration criteria cannot be met. Major changes include, but are not limited to, new columns or pump seals. A new calibration is not required after minor maintenance.

10.5. With the exception of the circumstances delineated in policy CA-Q-P-003, it is not acceptable to remove points from a calibration curve. At least seven points must be included in the calibration curve. Average Response Factor and linear fit calibrations

require a minimum of five points, whereas Quadratic (second order) calibrations require a minimum of six points.

10.6. A fixed injection volume is used for quantitation purposes and is to be the same for both the sample and standards.

10.7. All units used in the calculations must be consistently uniform, such as concentration in ng/mL.

10.8. Initial Calibration

10.8.1. A number of analytical standards of different analyte concentrations are used to generate the curve. Each standard is injected once to obtain the peak response for each analyte at each concentration. These standards define the working range of the analysis.

10.8.2. A minimum of five analytical standards is used when using average response factor and/or linear calibration fits.

10.8.3. A minimum of six analytical standards is used when a quadratic fit is used to generate the curve.

10.8.4. Calibration is by average response factor, linear fit, or by quadratic fit. Quadratic fit is used for the analyte if the response is non-linear.

10.8.5. For average response factor (RFa), the relative standard deviation (RSD) for all compounds quantitated against an identically labeled analog must be < 25% for the curve to be valid.

10.8.6. For linear fit, the intercept of the line must be less than ½ the reporting limit, and the coefficient of determination (r^2) must be greater than or equal to 0.990 for the curve to be considered valid (or the correlation coefficient (r) > 0.995).

10.8.7. The Internal Standard (IS) response (peak area) must not deviate by more than 50% from the average response (peak area) of the initial calibration.

10.9. Calibration Curve Fits

10.9.1. Linear regression or quadratic curves may be used to fit the data to a calibration function. Detailed descriptions and formulas for each fitting type can be found in SOP CA-Q-P-003, “Calibration Curves and Selection of Calibration Points”.

10.9.2. The linear curve uses the following function:

Equation 1

$$y = bx + c$$

Where:

y	=	**Error! Objects cannot be created from editing field codes.**
x	=	concentration
b	=	slope
c	=	intercept

10.9.3. The quadratic curve uses the following function:

Equation 2

$$y = ax^2 + bx + c$$

Where y, x, b, and c are the same as above, and a = curvature.

10.9.4. Evaluation of Calibration Curves

The following requirements must be met for any calibration to be used:

- Response must increase with increasing concentration.
- The absolute value of the intercept of a regression line (linear or non-linear) at zero response must be less than the reporting limit.
- There should be no carryover at or above 1/2 MRL after a high CAL standard.

If these criteria are not met, instrument conditions and standards will be checked, and the ICAL successfully repeated before continuing.

10.9.5. Weighting of Calibration Points

In linear and quadratic calibration fits, the points at the lower end of the calibration curve have less absolute variance than points at the high concentration end of the curve. This can cause severe errors in quantitation at the low end of the calibration. Because accuracy at the low end of the curve is very important for this analysis, it is preferable to increase the weighting of the lower concentration points. 1/concentration or 1/x weighting is encouraged. Visual inspection of the line fitted to the data is important in selecting the best fit.

10.10. Initial Calibration Blank (ICB)

10.10.1. Immediately following the ICAL, a calibration blank is analyzed that consists of an injection of [redacted] blank containing both IDA and IS.

10.10.2. The result for the calibration blank must be less than the reporting limit.

10.10.3. If the ICB is greater than the reporting limit then the source of contamination must be identified and any necessary cleaning completed, and then the instrument should be recalibrated.

10.11. Initial Calibration Verification (ICV)

10.11.1. Following the ICAL and the ICB, an ICV standard obtained from a different source or vendor than the ICAL standards is analyzed. This ICV standard is a mid-range standard.

10.11.2. The recovery for the ICV must meet the appropriate following criteria:

10.11.2.1. The native analyte must be within or equal to 50-150% for all native analytes quantitated against a closely related labeled analog IDA.

10.11.2.2. The IDA must be within or equal to 50-150%.

10.11.2.3. See Section 9.6 for corrective actions in the event that the ICV does not meet the criteria above.

10.12. Continuing Calibration Verification (CCV)

Analyze a CCV at the beginning of a run, the end of a run, and after every 10 samples to determine if the calibration is still valid. The exception is after an acceptable curve and ICV are run 10 samples can be analyzed before a CCV is required. The CCVs are usually at the mid-level range of the curve and should vary throughout the run from low level (LOQ/RL) to mid level. The curve and ICV do not need to be run every day. To start an analytical run a CCV can be analyzed and if it meets acceptance criteria a run can be started. In addition, the low standard in the curve must be analyzed and must be within ± 50% of the expected value.

10.12.1. The recovery for the CCV standards must be equal to or within 60 - 140% for all natives quantitated against an identically labeled analog and equal to or within 50 - 150% for all natives quantitated against a closely related labeled analog. The recovery for the IDA must be within or equal to 50 - 150%.

10.12.2. The Internal Standard (IS) response (peak area) must be within ± 50% from the response (peak area) from the midpoint of the initial calibration.

10.12.3. Sample IS response (peak area) must be within ± 50% of the response (peak area) in the most recent CCV.

10.12.4. If this is not achieved, the instrument has drifted outside the calibration limits.

The instrument must be recalibrated.

11. PROCEDURE

11.1. One-time procedural variations are allowed only if deemed necessary in the professional judgment of a supervisor to accommodate variation in sample matrix, chemistry, sample size, or other parameters. Any variation in procedure shall be completely documented using a con-conformance memo (NCM). The NCM process is described in more detail in SOP WS-QA-0023. The NCM shall be filed in the project file and addressed in the case narrative. Any deviations from this procedure identified after the work has been completed must be documented in an NCM, with a cause and corrective action described.

11.2. Solvent Dilution/Direct Injection (SDI) Procedure

The following procedure can be used for samples of known high concentration or of unknown levels that require screening to determine an appropriate aliquot size for the SPE extraction.

11.2.1. Mix the sample thoroughly in its original container and subsample a 0.5 mL aliquot.

11.2.2. Add 0.5 mL of Table 6 IDA solution and 0.5 mL of Table 6 IS to the 0.5 mL aliquot.

11.2.3. Prepare MB and LCS/LCSD aliquots similarly.

11.2.4. Dilute the sample aliquot to 10 mL FV in [redacted] by adding [redacted].

11.2.5. Prepare TB6 calibration standards at concentrations listed in Section 7.7 in [redacted].

11.2.5.1. [redacted].

11.2.6. Proceed to Section 11.7.4

11.2.7. Follow Sections 11.8 and 12 for extract analysis and data processing.

Note: The nominal calibration range for this procedure is 1-500 ug/L..

11.3. Water Sample Preparation

11.3.1. Visually inspect samples for the presence of settled and/or suspended

sediment/particulates. If present or if the sample is biphasic add IDA prior to any sample decanting or centrifugation. If the sample requires decanting or centrifugation contact the client for guidance prior to such action. Decanting or filtering of the sample can lead to a low bias.

11.3.2. If authorized by the client to filter the sample, filter the water sample through a glass fiber filter (Whatman GF/F Cat No 1825 090 or equivalent). Gravity or vacuum can be used to pass the sample through the filter. Prepare a filtration blank with any samples requiring filtration. File an NCM noting the need for filtration.

Warning: The use of a vacuum system creates the risk of glassware implosion. Inspect all glassware prior to use. Glassware with chips, scratches, rub marks or cracks must not be used.

11.3.3. Weigh the sample container prior to extraction and then weigh the sample container after extraction to determine the initial volume. Unless otherwise directed by client, use the entire sample volume.

11.3.4. Prepare additional aliquots of a field sample for the MS/MSD, if requested.

11.3.5. Prepare two 250 mL aliquots of HPLC-grade water for the method blank and LCS.

11.3.6. Spike the LCS and MS/MSD (if requested) with 0.5 mL of the LCS/Matrix Table 6 Spike solutions (Section 7.6). This will result in a sample concentration of 20 ng/L.

11.3.7. Add 0.5 mL of the Table 6 IDA solution (Section 7.7) into each sample and QC sample, for a fixed concentration of 1.25 ng/mL in the final sample vial.

11.4. Solid Phase Extraction (SPE) of Aqueous Samples

***Note**: The automated Zymark Auto-Trace Workstation can be used as long as the program follows these conditions and passes the background check.*

11.4.1. Condition the SPE cartridges ([redacted]) by passing the following without drying the column.

***Note:** The cartridges should not be allowed to go dry until the final elution step with [redacted]. At all of the other transition steps, the solvent/sample level should be stopped at the top of the column before the next liquid is added.*

WARNING: The use of a vacuum system creates the risk of glassware implosion. Inspect all glassware prior to use. Glassware with chips, scratches, rub marks or cracks must not be used.

11.4.2. Wash with 15.0 mL of [redacted].

11.4.3. Wash with 10.0 mL of [redacted]. Close valve when approximately 200 µL remains on top to keep column wet. After this step, the columns cannot go dry until the completion of loading and rinsing samples.

11.4.4. Appropriately label the columns and add the reservoir to the column.

11.4.5. Add samples to the columns and with vacuum, pull the entire 250 mL aliquot of the sample through the cartridge at a rate of approximately 2 to 5 drops per second.

11.4.5.1. If the SPE column should plug (flow rate <1 drop per minute) prior to the entire content of the sample container passing through the column do the following:

1. Stop adding sample to the reservoir.
2. Return any remaining sample volume back to the original container.
3. Weigh the original container and record this weight into the worksheet notes field within the TALS extraction batch.
4. Determine the full volume of sample fortified by using the "Gross Weight" – (remaining sample volume – default tare weight of a sample container (26.1 g)).
5. Enter this value into the "Initial Amount" field in the TALS extraction batch.
6. Proceed to Section 11.4, noting that additional vacuum or pressure might be needed to elute the SPE column.

11.4.6. After the sample completely passed through the cartridge, allow the column to dry well with vacuum for 10 minutes.

11.5. SPE Elution of Aqueous Samples – using 15 mL polypropylene test tubes as receiving tubes in the SPE manifold.

11.5.1. Rinse sample bottles with 4 mL of [redacted] and transfer to the column reservoir onto the cartridge. Allow the solution to soak for 5 minutes and then elute into the 15 mL collection tube.

11.5.2. Repeat sample bottle to column reservoir rinse and cartridge elution with a

second 4 mL aliquot of [redacted]. The total collection should be approximately 8 mL.

11.6. Extract Concentration

11.6.1. Fortify each sample with 2 mL of [redacted],

11.6.2. Seal the test tube tightly then vortex.

11.6.3. Concentrate the extract volume under a gentle stream of nitrogen in a water bath at 55ºC to approximately 1.5 mL.

11.7. Final volume for extracts

11.7.1. Bring sample to 2 mL using [redacted].

11.7.2. Add 0.5 mL of IS (0.025 g/mL) concentration.

11.7.3. Adjust the final volume of the extract to 10 mL using [redacted]. This will create an extract with a final solvent composition of [redacted].

11.7.4. Transfer a portion of the extract to a 300 µL polypropylene autosampler vial (7 drop-wise or approximately ½ filled is sufficient). Archive the rest of the extracts for re-injection and dilution.

11.7.5. Seal the vial with a polypropylene screw cap.

Note: *Teflon lined caps cannot be used due to detection of low level concentration of PFAS.*

11.8. Instrument Analysis

Suggested operating conditions are listed in Tables 1-3 for the SCIEX LCMS system.

Table 1				
Recommended Instrument Operating Conditions				
HPLC Conditions (Shimadzu HPLC)				
Column (Column temp = [redacted])	[redacted]			
Mobile Phase Composition	**A =** [redacted] **B =** [redacted]			
Gradient Program	**Time**	**%A**	**%B**	**Flow Rate - mL/min**
	0	90	10	0.30
	0.10	90	10	0.30
	2.00	80	20	0.30
	2.50	45	55	0.30

Table 1				
Recommended Instrument Operating Conditions				
HPLC Conditions (Shimadzu HPLC)				
Column (Column temp = [redacted])	[redacted]			
Mobile Phase Composition	**A** = [redacted]		**B** = [redacted]	
	5.00	5	95	0.30
	9.45	1	99	0.30
	9.50	90	10	0.30
	10.50	90	10	0.30
	Maximum pressure limit = 7,500 psi			
Injection Size	20 µL (fixed amount throughout the sequence).			
Run Time	~13 minutes			
Mass Spectrometer Interface Settings (SCIEX 5500)				
MS Interface Mode	ESI Negative Ion. Minimum of 10 scans/peak.			
Ion Spray Voltage (kV)	4.5			
Entrance Potential (V)	5			
Declustering Potential (V)	25			
Desolvation Temp	550ºC			
Curtain Gas	35 psi			
Collision Gas	8 psi			

Table 2

Recommended Instrument Operating Conditions

Mass Spectrometer Scan Settings (SCIEX 5500)

Compound	Reaction (MRM)	Dwell (sec)	Ent. Pot. (V)	Col. Energy (V)	Declu. Pot. (V)	Cell Exit Pot. (V)	Typ RT (Min)
PMPA	229 > 185	0.011	-10	-12	-15	-5	6.47
PEPA	278.9 > 234.9	0.011	-10	-10	-20	-5	7.07
DFSA	174.9 > 81	0.011	-10	-32	-25	-7	1.3
MMF	139 > 51	0.011	-10	-20	-20	-9	1.34
MTP	175 > 97	0.011	-10	-22	-45	-9	2.3
PPF Acid	163 > 118.9	0.011	-10	-16	-35	-13	3.12
13C4 PFBA	217 > 172	0.011	-5	-12	-25	-31	6.3
13C2 PFOA	415 > 370	0.011	-6	-14	-25	-44	8.02

Table 3

Analyte Retention Times	
Table 6 Compounds	**Typical Native RT (minutes)**
PEPA	7.07
PMPA	6.47
DFSA	1.30
MMF	1.34
MTP	2.30
PPF Acid	3.12
13C4PFBA	6.30
13C2-PFOA	8.02

11.8.1. Tune and calibrate the instrument as described in Section 10.

11.8.2. A typical run sequence is as follows:

- Rinse Blank (RB, not linked to anything)
- Start ICAL with CCVL but called IC in TALS (starts the 12 hour clock or time 0:00)
- Rest of ICAL
- ICB: link to midpoint of ICAL and samples
- ICV: link to midpoint of ICAL and samples (If ICAL good)
- CCB: link to midpoint of ICAL and samples
- PFOA RT marker
- Rinse Blank (RB, not linked to anything)
- 10 samples: link to midpoint of ICAL
- CCV: link to midpoint of ICAL
- 10 more samples: link to midpoint of ICAL
- CCV: link to midpoint of ICAL
- Etc.

12. CALCULATIONS

12.1. If the concentration of the analyte ions exceeds the working range as defined by the calibration standards, then the sample might require to be diluted and reanalyzed, based upon client need. It may be necessary to dilute samples due to matrix.

12.2. Matrix spike samples that exceed the working range when the unspiked sample is in the range are reported without further dilution and flagged appropriately.

12.3. Qualitative Identification

The analyte RT must be within ± 0.3 minutes of the ICV and CCV standards.

12.4. The ICAL established in Section 10 is used to calculate concentrations for the extracts.

12.5. Extract concentrations are calculated as below. Each equation applies to a different calibration model, as noted.

Equation 3

Equation 4

$$\text{Concentration, ng/mL} = \frac{y - c}{b}$$

Equation 5

$$\text{Concentration, ng/mL} = \frac{-b + \sqrt{b^2 - 4a(c - y)}}{2a}$$

Where:

y	=	Area (analyte)
CF	=	Calibration Factor (average response factor model only)
a	=	curvature
b	=	slope
c	=	intercept

12.6. Water Sample Result Calculation:

Equation 6

$$\text{Concentration, ng/L} = \frac{C_{ex} V_t}{V_o}$$

Where:

C_{ex}	=	Concentration measured in sample extract (ng/mL)
V_t	=	Volume of total extract (mL)
V_o	=	Volume of water extracted (L)

12.7. IDA Recovery Calculation:

Equation 7

$$\%\,\text{Recovery} = \frac{A_t Q_{is}}{A_{is} Q_t RRF_{IDA}} X100$$

Where ng/g = µg/kg and:

RF_{IDA}	=	Response Factor for IDA compound
A_t	=	Area response for IDA compound
A_{IS}	=	Area Response for IS compound
Q_{IS}	=	Amount of IS added

Q_t = Amount of IDA added

12.8. Raw data, calibration summaries, QC data, and sample results are reviewed by the analyst. These must also be reviewed thoroughly by a second qualified person. See the Data Review Policy (WS-PQA-0012). These reviews are documented on the Data Review Checklist.

13. METHOD PERFORMANCE

13.1. The group/team leader has the responsibility to ensure that this procedure is performed by an associate who has been properly trained in its use and has the required expertise.

13.2. Method Detection Limit

The laboratory must generate a valid method detection limit for each analyte of interest. The MDL must be below the reporting limit for each analyte. The procedure for determination of the method detection limit is given in 40 CFR Part 136, Appendix B, and further defined in SOP WS-QA-0006 and policy WS-PQA-003. MDLs are available in the Quality Assurance Department.

13.3. Initial Demonstration of Capability (IDOC)

Each analyst performing this procedure must successfully analyze four LCS QC samples using current laboratory LCS control limits. IDOCs are approved by the Quality Assurance Manager and the Technical Director. IDOC records are maintained by the QA staff in the central training files.

13.4. The laboratory must generate a valid method detection limit for each analyte of interest. The MDL must be below the reporting limit for each analyte. The procedure for determination of the method detection limit is given in 40 CFR Part 136, Appendix B, and further defined in WS-QA-0006 and policy WS-PQA-003.

14. POLLUTION PREVENTION

14.1. All waste will be disposed of in accordance with Federal, State and Local regulations.

14.2. Standards and reagents are purchased and prepared in volumes consistent with laboratory use to minimize the volume of expired standards and reagents requiring disposal.

14.3. Where reasonably feasible, technological changes have been implemented to minimize the potential for pollution of the environment. Employees will abide by this method and the policies in Section 13 of the Corporate Safety Manual for “Waste Management and Pollution Prevention.”

14.4. Do not allow waste solvent to vent into the hoods. All solvent waste is stored in capped containers unless waste is being transferred.

14.5. Transfer waste solvent from collection cups (tri-pour and similar containers) to jugs and/or carboys as quickly as possible to minimize evaporation.

15. WASTE MANAGEMENT

The following waste streams are produced when this method is carried out:

15.1. Assorted test tubes, autovials, syringes, filter discs and cartridges. Dump the solid waste into a yellow contaminated lab trash bucket. When the bucket is full or after no more than one year, tie the plastic bag liner shut and put the lab trash into the hazardous waste – landfill steel collection drum in the H3 closet. When the drum is full or after no more than 75 days, move it to the waste collection area for shipment.

15.2. Waste [redacted]. Collect the waste solvents in tripours during use. Empty the tripours into a 1-liter to 4-liter carboy at the fume hood. When the carboy is full, or after no more one year, whichever comes first, empty the carboy into the steel flammable solvent drum in the H3 closet. When full to no less than six inches of the top, or after no more than 75 days, move the steel flammable solvent drum to the waste collection area for shipment.

15.3. Aqueous acidic waste from the LCMS instrument contaminated with [redacted]. This is collected in a 1-gallon carboy at the instrument. When the carboy is full, or after no more than one year, whichever comes first, it is emptied into the blue plastic HPLC collection drum in the H3 closet. When the drum is full to between two and six inches of the top, or after no more than 75 days, move it to the waste collection area for shipment.

15.4. Autovials contaminated with [redacted]. As the autovials are removed from the instrument after analysis, they are collected in open containers at the instrument. After all autovials are removed, the open container must be dumped into a closed satellite collection container in a fume hood, as the punctured septa in the autovial can allow [redacted] and other contaminants to evaporate into the atmosphere. The satellite collection containers are transferred to the waste disposal area when full or after no more than one year, whichever comes first, where they are disposed through the vial eater.

16. REFERENCES

16.1. A. Petlick edit of J Boyle, “Determination of Table 3 Compounds by LC/MS/MS Chemours Fluoroproducts Analytical Method”, 4/3/2018.

16.2. A. Petlick, "Determination of Table 3 *Plus* Compounds by LC/MS/MS Chemours Fluoroproducts Analytical Method", 1/10/2019

17. METHOD MODIFICATIONS

17.1. Modifications from the reference methods are detailed below:

17.1.1. Analytical columns are specific to TestAmerica's inventory.

17.1.2. The LCMS system is specific to TestAmerica's fleet.

17.1.3. The entire sample (250 mL) is extracted via SPE.

17.1.4. Target analytes are quantitated via isotope dilution.

17.1.5. A labeled analyte is used to monitor extraction efficiency and instrument performance.

18. ATTACHMENTS

18.1. None

19. REVISION HISTORY

19.1. WS-LC-0033, Revision 1.1, Effective 05/26/2020

19.1.1. Table 1.2 revised, "2 ng/L" to "2 ng/L – 4 ng/L" and "2 ng/L – 80 ng/L" to "2 ng/L – 2000 ng/L".

19.1.2. Editorial changes.

19.2. WS-LC-0033, Revision 1.0, Effective 05/22/2020

19.2.1. Table 5.2 revised, "250 ppm (STEL)" added to [redacted] exposure limit. NFPA rating updated for [redacted].

19.2.2. Section 7.4 revised, [redacted].

19.2.3. Table 7.7.4 revised, expanded calibration concentration range.

19.2.4. Inserted Section 11.2, "Solvent Dilution/Direct Injection (SDI) Procedure" and its associated subsections.

19.2.5. Editorial changes.

19.3. WS-LC-0033, Revision 0, Effective 03/26/2020

19.3.1. This is the initial version of this SOP.