CAC-TFA-1.0
Determination of Trifluoroacetate in Drinking Water by Liquid Chromatography / Tandem Mass Spectrometry (LC/MS/MS)

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Determination of Trifluoroacetate in Drinking Water by Liquid Chromatography / Tandem Mass Spectrometry (LC/MS/MS)

1. Scope:

This is a direct aqueous injection liquid chromatography / tandem mass spectrometry (LC/MS/MS) method for the determination of trifluoroacetate (TFA) in drinking water. TFA is an ultrashort-chain perfluoroalkyl substance (PFAS). This test is validated for use on drinking water samples pre- and post-treatment at water treatment plants, with a single laboratory Minimum Reporting Limit of 0.085 ppb.

2. Principle:

A 1 mL aliquot of the sample is spiked with an isotopically labelled internal standard prior to analysis by LC/MS/MS. The internal standard serves to correct for variations in instrument response and matrix effects, ensuring accurate quantification. A 50 µL injection of the prepared sample is introduced into an LC system equipped with a hydrophilic interaction chromatography (HILIC) column. Identification of TFA is achieved by comparing the mass spectra and retention times to the reference data for the calibration standards. The concentration of TFA is calculated with the internal standard technique.

3. Definitions:

3.1. ANALYSIS BATCH – A set of samples that are analyzed on the same instrument during a 24-hour period that begins and ends with the analysis of the appropriate Continuing Calibration Check (CCC) standards. Additional CCCs may be required depending on the length of the Analysis Batch and the number of field samples.

3.2. CALIBRATION STANDARDS – Solutions of TFA that are prepared from the Primary Dilution Standards. The calibration standards are used to calibrate the instrument response with respect to analyte concentration.

3.3. CONTINUING CALIBRATION CHECK (CCC) – Solutions of TFA that are prepared from the Primary Dilution Standards. They are analyzed to verify batch calibration. CCCs are run at the beginning of each Analysis Batch, after every ten field samples, and at the end of the Analysis Batch. The first CCC must be at the MRL to verify initial instrument sensitivity. Subsequent CCCs should alternate between mid- and high-level CAL standards. The calibration standards may be used as CCCs.

3.4. FIELD DUPLICATES (FD1 and FD2) – Two separate samples collected at the same time, placed under identical circumstances,

and treated exactly the same throughout field and laboratory procedures. Analyses of FD1 and FD2 give a measure of the precision associated with sample collection, preservation, and storage, as well as laboratory procedures.

3.5. INTERNAL STANDARD (IS) – A pure compound that is added to all standard solutions and samples in a known amount and used to measure the relative response of other method analytes that are components of the same solution. The internal standard must respond similarly to the method analyte, have no potential to be present in water samples, and not be a method analyte.

3.6. LABORATORY FORTIFIED BLANK (LFB) - An aliquot of reagent water fortified with a known quantity of TFA. The LFB is prepared to match the analytical procedure for field samples. The LFB is used during the IDC to verify method performance for precision and accuracy. The LFB is also a required QC element with each Analysis Batch. The results of the LFB verify method performance in the absence of sample matrix.

3.7. LABORATORY FORTIFIED SAMPLE MATRIX (LFSM) - An aliquot of a field sample fortified with a known quantity of TFA. The purpose of the LFSM is to determine the bias contribution of the sample matrix to the analytical results.

3.8. LABORATORY FORTIFIED SAMPLE MATRIX DUPLICATE (LFSMD) – A second aliquot of the same field sample used to prepare the LFSM, fortified and analyzed in the same Analysis Batch as the LFSM. The LFSMD is used to verify method precision in sample matrices.

3.9. LABORATORY REAGENT BLANK (LRB) - An aliquot of reagent water prepared to match the sample processing procedures. The LRB is used to check if TFA or other interferents are introduced from sample containers, processing equipment, or the reagents of the assay.

3.10. LOWEST CONCENTRATION MINIMUM REPORTING LEVEL (LCMRL) - The single-laboratory LCMRL is the lowest spiking concentration such that the probability of spike recovery in the 50 to 150% range is at least 99%.

3.11. MINIMUM REPORTING LEVEL (MRL) - The minimum concentration that can be reported by a laboratory as a quantified value for TFA in a sample following analysis. This concentration

must meet the criteria defined in Section 9.1.3 and must be no lower than the concentration of the lowest calibration standard.

3.12. PRIMARY DILUTION STANDARD (PDS) - A solution of TFA in acetonitrile. PDS solution are made from Stock Standard solutions. The PDS solutions are used to fortify the QC samples.

3.13. QUALITY CONTROL SAMPLE (QCS) - A solution containing TFA at a known concentration that is obtained from a source different from the source of calibration standards. The purpose of the QCS is to verify the accuracy of the primary calibration standards.

3.14. REAGENT WATER - Purified water that does not contain any measurable quantity of TFA or interfering compounds at or above 1/3 the MRL.

3.15. STOCK STANDARD SOLUTION - a concentrated standard that is purchased from a commercial source with a certificate of analysis.

4. Interferences:

Method interferences may be caused by contaminants in solvents, reagents (including reagent water), sample bottles and caps, and other sample processing hardware that lead to discrete artifacts or elevated baselines in the chromatograms. The analytes in this method can also be found in many common laboratory supplies and equipment, such as PTFE (polytetrafluoroethylene) products, LC solvent lines, methanol, etc. Laboratories must demonstrate that these items are not contributing to interference by analyzing LRBs as described in Section 9.2.1.

Aqueous samples should not come in contact with any glass containers or pipettes as PFAS analytes can potentially adsorb to glass surfaces. Standards dissolved in organic solvent may be purchased in glass ampoules. These standards in organic solvent are acceptable and subsequent transfers may be performed using glass syringes and pipets.

5. Safety:

5.1. Read the Safety Data Sheet for all materials before use.
5.2. All general laboratory safety rules for sample preparation and analysis shall be followed.
5.3. All solvents should be handled with care in a ventilated area.

6. Equipment and Supplies:

6.1. Micropipettes with disposable tips (10 – 1000 µL)
6.2. Multi-channel, stepper, or electronic repeating pipette with disposable tips
6.3. Vortex vibrating mixer.
6.4. Polypropylene (PP) autosampler vials (1.5mL) with silicon lined caps.
6.5. Liquid Chromatography Tandem Mass Spectrometry System (LC/MS/MS)

6.5.1. LC System

The LC system must provide consistent sample injection volumes and be capable of performing binary linear gradients at a constant flow rate.

6.5.2. Analytical Column

This method was developed using a Phenomenex bioZen Glycan 2.6 µm, 150 x 2.1 mm column (00F-4773-AN). Any column that provides adequate resolution, peak shape, capacity, accuracy and precision (Sect. 9), and does not exacerbate suppression or enhancement of analyte responses may be used.

6.5.3. Tandem Mass Spectrometer

The mass spectrometer must be capable of electrospray ionization. The system must be capable of performing MS/MS to produce unique product ions for the method analytes within specified retention time segments. A minimum of 10 scans across the chromatographic peak is needed to ensure adequate precision.

6.5.4. MS/MS Data System

An interfaced data system is required to acquire, store, and output MS data. The computer software must have the capability of processing stored data by recognizing a chromatographic peak within a given retention time window. The software must allow integration of the abundance of any specific ion

between specified time or scan number limits. The software must be able to construct a linear regression or quadratic regression calibration curve and calculate analyte concentrations using the internal standard technique.

7. Reagents and Standards:

7.1. Acetonitrile, Fisher Optima or equivalent
7.2. Isopropyl alcohol
7.3. Formic acid
7.4. Ammonium formate
7.5. Aqueous Mobile Phase during method development: water with 10mM ammonium formate and 0.1% formic acid
7.6. Organic Mobile Phase during method development: acetonitrile/isopropyl alcohol (95/5 v/v) with 0.1% formic acid
7.7. Rinse solution 0/1: 80/20 water/MeOH, 0.1% formic acid
7.8. Rinse solution 2: MeOH
7.9. Rinse solution 3: 90/10 ACN/water, 0.1% formic acid
7.10. Method Analyte Standard
Trifluoroacetic acid, sodium salt CAS# 2923-18-4
Cambridge Isotopes Labs ULM-11087-1.2 used during method development. Primary dilution standards (PDS) are made by dilution in acetonitrile. PDS expire at the same time as the stock standards, follow manufacturer recommendations. Check PDS for performance every 6 months until expiration.
7.11. Internal Standard
Trifluoroacetic acid, sodium salt ($^{13}C_2$, 99%) CAS# N/A
Cambridge Isotopes Labs CLM-11046-1.2 used during method development. PDS are made by dilution in acetonitrile. PDS expire at the same time as the stock standards, follow manufacturer recommendations. Check PDS for performance every 6 months until expiration.
7.12. Calibration Standards
Prepare a series of calibration standards of at least five levels by serial diluting the analyte PDS into reagent water. The lowest calibration standard must be at or below the MRL for each analyte. The calibration standards may also be used as Continuing Calibration Checks (CCCs). Using the PDS solutions, add a constant amount of the internal standard to each calibration standard. The concentration of the internal standard should match the concentration of the internal standard in samples.

8. Sample Collection, Preservation and Storage:

8.1. Samples should be collected in amber high-density polyethylene (HDPE) bottles, with polypropylene or HDPE screw caps. Discard sample bottles after a single use.

8.1.1. Samplers must wash hands before sampling and wear nitrile gloves while filling and sealing the sample bottles, using a new pair of nitrile gloves at each sample site.

8.1.2. To collect the sample, open the tap and allow the system to flush until the water temperature has stabilized or allowed to flow for a minimum of 15 minutes before sampling to ensure that the sample reflects the water quality of the source. Collect the samples from the flowing system. After collecting the sample, cap the bottle and place the sample bottles into the ice chest with wet ice and keep them cool from time of collection until extraction.

8.2. FIELD REAGENT BLANKS (FRB) – Each sample set must include an FRB. A sample set is defined as samples collected from the same site and at the same time.

8.2.1. Reagent water used for the FRBs must be analyzed prior to shipment to ensure the water has minimal residual TFA. Extract an LRB prepared with reagent water using the same lot of sample bottles destined for shipment to the sampling site and ensure that analyte concentrations are less than one-third the MRL, as described in Section 9.2.1. This will ensure that any significant contamination detected in the FRBs originated from exposure in the field.

8.2.2. In the laboratory, fill the FRB sample bottle with the analyzed reagent water (Sect. 8.4.1), then seal and ship to the sampling site with the sample bottles. For each FRB shipped, a second empty FRB sample bottle must also be shipped. At the sampling site, open the FRB bottle and pour the reagent water into

the second sample bottle; seal and label this bottle as the FRB with the date, time and location of the site.

8.2.3. The FRB must be collected prior to the collection of the field sample and field duplicate

8.2.4. The FRB is treated as a sample in all respects, including shipment to the sampling site, exposure to sample conditions, storage, and all analytical procedures

8.3. SAMPLE SHIPMENT AND STORAGE – Samples must be chilled during shipment. Samples must be confirmed to be at or below 10 °C when they are received at the laboratory. In the laboratory, samples must be stored at or below 4 °C and protected from light. Samples must not be frozen

8.4. SAMPLE HOLDING TIMES – Analyze samples as soon as possible. Samples that are collected and stored as described in Section 8 must be analyzed within 28 days of collection.

9. Quality Control:

9.1. INITIAL DEMONSTRATION OF CAPABILITY (IDC)

9.1.1. DEMONSTRATION OF PRECISION AND ACCURACY - Prepare seven replicate LFBs, fortified with TFA near the midpoint of the laboratory's calibration curve. LFBs must be processed in a single Analysis Batch. The Analysis Batch should also include the LRBs from section 9.1.2, as well as a low Continuing Calibration Check (CCC) (see 10.5). The percentage relative standard deviation (%RSD) for the LFBs must be ≤15%. The mean recovery for the LFBs must be ≥70% and ≤130%.

9.1.2. DEMONSTRATION OF LOW SYSTEM BACKGROUND - Included in the Analysis Batch in section 9.1.1, prepare five LRBs. The results for each LRB must be less than one-third the MRL.

9.1.3. MINIMUM REPORTING LIMIT (MRL) CONFIRMATION – The suggested Minimum Reporting Limit (MRL) for TFA by this method has been set at 0.085 ppb. EPA methods require that the MRL be confirmed by analyzing seven samples spiked at the proposed MRL. The Analysis Batch for

the MRL confirmation must include two LRBs and a low CCC. The results of these spikes must meet the following requirements for the Prediction Interval of Results (PIR). If the criteria are not met, the MRL should be raised and the confirmation step repeated until it passes.

Half Range=3.963*S, where S is the standard deviation and 3.963 is a constant for seven replicates.

Upper PIR Limit=$\frac{Mean+HR}{Spiked\ Conc.} x100\% \leq 150\%$

Lower PIR Limit=$\frac{Mean-HR}{Spiked\ Conc.} x100\% \geq 50\%$

9.1.4. QUALITY CONTROL SAMPLE (QCS) - Analyze a mid-level QCS prepared as in section 9.2.8, to confirm the accuracy of the calibration standards.

9.2. ANALYSIS BATCH QC REQUIREMENTS

9.2.1. LABORATORY REAGENT BLANK (LRB) - For each Analysis Batch, include one LRB. The TFA concentration must be less than one-third the MRL. If the concentration is greater than or equal to that level, any positive results from that Analysis Batch are invalid.

9.2.2. CONTINUING CALIBRATION CHECK (CCC) - Analyze CCC standards at the beginning of each Analysis Batch, after every ten field samples, and at the end of the Analysis Batch. See Section 10.5 for concentration requirements and acceptance criteria for CCCs.

9.2.3. LABORATORY FORTIFIED BLANK (LFB) - A LFB must be included in each Analysis Batch. The concentration of the LFB must be rotated between low, medium, and high concentrations from batch to batch. The percent recovery for each LFB must be ±50% of the true value if spiked at less than 2 times

the MRL or ±30% if greater than 2 times the MRL, else the entire Analysis Batch is invalid.

9.2.4. INTERNAL STANDARDS (IS) - The analyst must monitor the peak areas of the internal standards in all injections of the Analysis Batch. The internal standard responses (as indicated by peak areas) for any chromatographic run must not deviate by more than ±40% from the average areas measured during the initial calibration for the internal standards. If the IS areas in a chromatographic run do not meet these criteria, check the corresponding IS of the most recent CCC and proceed as follows:

9.2.4.1 IS Failure in Sample but not CCC If the IS criterion is met in the CCC but not in the sample, reanalyze the sample in the same or subsequent Analysis Batch. If the reanalyzed sample produces an acceptable IS response, report results for that injection. If the IS area count fails to meet the acceptance criterion in the repeated analysis but still passes the most recent CCC, report the sample results as “suspect/matrix.” Alternatively, analyze a FD or collect a new sample and reanalyze.

9.2.4.2 IS Failure in Sample and CCC If both the original sample and the CCC fail the IS criteria, take corrective action (e.g., Sect. 10.6). It might be helpful to check the integrity of the IS solution and the fortification technique before reanalyzing the sample in a subsequent Analysis Batch. After corrective action, re-inject the sample in a subsequent Analysis Batch. If the IS area fails to meet the acceptance criterion in the repeat analysis, but passes in the most recent

CCC, report the sample results as "suspect/matrix."

9.2.5. LABORATORY FORTIFIED SAMPLE MATRIX (LFSM) / LABORATORY FORTIFIED SAMPLE MATRIX DUPLICATE - One set of LFSM and LFSMD is required with each Analysis Batch. One set is required for each 20 field samples present in the batch. The native background concentration must be determined from a separate field sample. The source of the sets should be distributed among the various water sources for the laboratory over time.

9.2.5.1 Three separate aliquots of a field sample are required, one to determine the native background concentration and one each for the LFSM and LFSMD. Homogenize the sample before separating into three vials. Fortify the LFSM and LFSMD greater than the native concentration of TFA in the sample. Selecting a duplicate aliquot of a sample that has already been analyzed aids in the selection of an appropriate spiking level. If this is not possible, use historical data when selecting a fortifying concentration.

9.2.5.2 Calculate the mean percent recovery for each LFSM and LFSMD set:

$$\%R = \frac{(A-B)}{C} \times 100\%$$

A= mean measured concentration of set
B= measured native background
C= fortification concentration

9.2.5.3 The mean percent recovery for each set must be ≤±50% of the true value if spiked at less than 2 times the MRL or ≤±30% if greater than 2 times the MRL. If the percent recovery is outside this range, and the performance of the LFBs is in control for the same batch, the

recovery may be matrix biased. Mark the result for the sample from which the LFSM was prepared as “suspect-matrix”.

9.2.5.4 Calculate the relative percent difference (RPD):

$$RPD = \frac{|LFSMD - LFSM|}{(LFSMD + LFSM)/2} \times 100\%$$

9.2.5.5 The RPD for each set must be ≤50% if spiked at less than 2 times the MRL, or ≤30% if greater than 2 times the MRL. If the RPD is outside this range, and the performance of the LFBs is in control for the same batch, the precision may be matrix biased. Mark the result for the sample from which the LFSMD was prepared as “suspect-matrix”.

9.2.6. FIELD DUPLICATES (FD) – If the method analyte is routinely observed in field samples, FD may be analyzed rather than LFSMD.

9.2.6.1 Calculate the relative percent difference (RPD) for duplicate measurements. (FD1 and FD2) using the equation:

$$RPD = \frac{|FD_1 - FD_2|}{(FD_1 + FD_2)/2} \times 100\%$$

9.2.6.2 RPDs for FD must be ≤50% if spiked at less than 2 times the MRL, or ≤30% if greater than 2 times the MRL. If the RPD of an analyte falls outside the designated range, and the laboratory performance for the analyte is shown to be in control in the CCC and in the LFB, the precision is judged matrix influenced. Report the result for the

corresponding analyte in the unfortified sample as “suspect–matrix”

9.2.7. RETENTION TIME (RT) – The retention time for each field sample in an Analysis Batch must be ±0.05 min from the RT of the calibration standards of that batch.

9.2.8. QUALITY CONTROL SAMPLE (QCS) - A QCS must be analyzed during the IDC, and again with each new set of calibration standards. The TFA used for the QCS must be procured from a source that is independent of the source of the Stock Standard. The concentration of the QCS should be near the center of the calibration curve. The percent recovery for the QCS must be ±30% of the true value.

10. Calibration and Standardization:

10.1. Mass Calibration

10.1.1. Calibrate the mass spectrometer as specified by the manufacturer.

10.2. MS/MS Optimization

10.2.1. Each LC/MS/MS system will have different optimal conditions, depending on source geometry and system design. Follow manufacturer recommendations for tuning the instrument.

10.2.2. During the development of this method, instrumental parameters were optimized for the precursor and product ions listed in Table 3. While the optimized conditions for these ions may vary, analysts are not permitted to use alternative ions for TFA due to its small size and lack of other easily identifiable fragments.

10.2.3. Optimize the response of the precursor and product ions for TFA and $^{13}C_2$-TFA according to manufacturer recommendations. The MS parameters used during method development are listed in Table 2. The

MS/MS parameters determined during method development are listed in Table 3.

10.3. Chromatographic Conditions

10.3.1. Establish LC method parameters to optimize peak shape. The LC parameters used during method development can be found in Table 1. Modifying conditions (i.e. mobile phase composition, and LC column) is allowed only if the QC criteria in Section 9 are still satisfied.

10.3.2. Steps should be taken to minimize TFA background from the LC system components and mobile phases. The column should be washed regularly to reduce TFA build up and carryover.

10.4. Initial Calibration

10.4.1. The calibration standard curve must contain at least 5 standards, the lowest calibration standard must be at or below the MRL. The method development calibration curve consisted of 8 levels: 0.04, 0.05, 0.075, 0.100, 0.500, 1.00, 10.0, and 20.0 ppb.

10.4.2. Fit the calibration points with either a linear or quadratic regression, calibration must be done using peak areas and the internal standard technique. During method development, a linear calibration curve weighted (1/X) was used.

10.4.3. Validate the initial calibration by calculating the concentration of TFA as an unknown at each calibration level. For calibration levels ≤MRL, results should be within ±50% of the true value. All other calibration levels should be within ±30% of their true values. The Relative Standard Error of the calibration must be ≤15%.

$$\%RSE = 100 \times \sqrt{\sum_{i=1}^{n} \left[\frac{x_i' - x_i}{x_i}\right]^2 / (n-p)}$$

x_i = True value of the calibration level *i*
x'_i = Measured concentration of calibration level i
p = Number of terms in the fitting equation
(average = 1, linear = 2, quadratic = 3)

n = number of calibration points

10.4.4. If validation of the initial calibration is not successful, reanalyze the calibration standards or restrict the calibration range. If the cause of failure is due to contamination or degradation of the calibration standards, prepare fresh calibration standards and repeat the initial calibration steps.

10.5. Continuing Calibration Checks (CCCs)

10.5.1. Analyze a CCC at the beginning of each Analysis Batch to verify the calibration, after every 10th sample, and at the end of the Batch. The first CCC must be at the MRL to verify initial instrument sensitivity. Subsequent CCCs should alternate between mid- and high-level CAL standards.

10.5.2. Verify that the peak areas of the quantitation ion of the internal standard have not changed by more than ±50% of the average areas measured in the initial calibration. If the internal standard peaks areas are not within the limits, see Section 10.6 for corrective actions.

10.5.3. Calculate the concentration for TFA in each CCC. Those fortified at the MRL must be ±50% of the true value. The mid- and high-calibration levels must be within ±30% of their true values. If the limits are exceeded, then any samples run since the last passing CCC must be reanalyzed after an acceptable calibration has been reestablished.

10.6. Corrective Action

10.6.1. Failure to meet CCC QC criteria necessitates corrective action. Performance may be restored by flushing the column with 100% ACN. After this or other minor corrective steps, check with calibration with both an MRL- and mid-level CCC. If failures continue, more major changes may be necessary, such as replacing the LC column or MS/MS system service. If major maintenance is performed, return to initial calibration (Section 10.4) before proceeding.

11. Procedure:

11.1. All samples, standards, and reagents are allowed to attain room temperature before the analysis.

11.2. Preparation of blank and fortified samples

11.2.1. LRB: spike 0.100 ppb internal standard (10 µL of 10 ppb stock standard) into 1 mL of reagent water. Cap vial and vortex to mix.

11.2.2. LFB / LFSM / LFSMD: spike a concentration greater than or equal to the native background concentration, if known, of TFA (or 0.100 ppb) into 1 mL of reagent water (or field sample / duplicate). Cap each vial and vortex to mix.

11.3. Test sample preparation.

11.3.1. Allow samples to come to room temperature.

11.3.2. Mix the sample within its container to ensure homogeneity. Aliquot 1 mL of each sample into a 1.5 mL polypropylene (PP) vial, return remaining samples to refrigerator for continued preservation.

11.3.3. Spike 0.100 ppb internal standard (10 µL of 10 ppb stock standard) into vials. Cap vial and vortex to mix.

11.4. Sample Analysis

11.4.1. Establish MS/MS operating conditions per the procedures in Section 10.2 and chromatographic conditions per Section 10.3. Establish a valid initial calibration following the procedures in Section 10.4 or confirm that the existing calibration is still valid by analyzing a low-level CCC. If establishing an initial calibration for the first time, complete the IDC prior to analyzing field samples. Analyze field and QC samples in a properly sequenced Analysis Batch as described in Section 11.5.

11.4.1. The analyst must ensure that the method analyte elutes entirely within the assigned window during each Analysis Batch. Make this observation by viewing the quantitation ion for each analyte in the

CCCs analyzed during an Analysis Batch. If an analyte peak drifts out of the assigned window, then data for the analyte is invalid in all injections acquired since the last valid CCC.

11.5. Analysis Batch Sequence

11.5.1. An Analysis Batch is a sequence of samples, analyzed within a 24-hour period, of no more than 20 field samples and includes all required QC samples (LRB, CCCs, the LFSM and LFSMD (or FD)). The required QC samples are not included in counting the maximum field sample total of 20. LC-MS/MS conditions for the Analysis Batch must be the same as those used during calibration.

11.5.2. ANALYZE INITIAL CCC – After a valid calibration is established, begin every Analysis Batch by analyzing an initial low-level CCC at or below the MRL. This initial CCC must be within ±50% of the true value for each method analyte and must pass the IS area criterion (Sect. 10.5.2). The initial CCC confirms that the calibration is still valid. Failure to meet the QC criteria may indicate that recalibration is required prior to analyzing samples. After the initial CCC, continue the Analysis Batch by analyzing an LRB, followed by field and QC samples at appropriate frequencies (Sect. 9.2). Analyze a mid- or high-level CCC after every ten field samples and at the end each Analysis Batch. Do not count QC samples (LRBs, FDs, LFSMs, LFSMDs) when calculating the required frequency of CCCs.

11.5.3. ANALYZE FINAL CCC – A final CCC completes the Analysis Batch. The acquisition start time of the final CCC must be within 24 hours of the acquisition start time of the initial low-level CCC at the beginning of the Analysis Batch. More than one Analysis Batch within a 24-hour period is permitted.

12. Data Analysis and Calculations:

12.1. ESTABLISH A RETENTION TIME WINDOW - Establish an appropriate retention time window for the analyte to identify them in the resulting chromatograms. Base this assignment on

measurements of actual retention time variation for each compound in standard solutions over the course of time. The suggested variation is plus or minus three times the standard deviation of the retention time for each compound for a series of injections. The injections from the initial calibration and from the IDC (Sect. 9.1) may be used to calculate the retention time window. However, the experience of the analyst should weigh heavily on the determination of an appropriate range.

12.2. IDENTIFY PEAKS OF INTEREST - At the conclusion of data acquisition, use the same software settings established during the calibration procedure to identify peaks of interest in the predetermined retention time windows. Confirm the identity of the analyte by comparison of its retention time with that of the corresponding analyte peak in an initial calibration standard or CCC.

12.3. CALCULATE ANALYTE CONCENTRATIONS - Calculate analyte concentrations using the multipoint calibration established in Section 10.4. Report only those values that fall between the MRL and the highest calibration standard.

12.4. ROUND CONCENTRATIONS - Calculations must use all available digits of precision, but final reported concentrations should be rounded to an appropriate number of significant figures (one digit of uncertainty), typically two, and not more than three significant figures.

12.5. EXCEEDING THE CALIBRATION RANGE - The analyst must not extrapolate beyond the established calibration range. If an analyte result exceeds the range of the initial calibration curve, the sample may be diluted using reagent water and the appropriate amount of internal standard added to match the original level. Reinject the diluted sample. Incorporate the dilution factor into final concentration calculations. The resulting data must be annotated as a dilution, and the reported MRLs must reflect the dilution factor.

13. Method Performance:

13.1. EPA’s Alternative Testing Procedure (ATP) protocol was followed, where possible, for method development and validation.

13.2. Method Detection Limits (MDL) refers to the lowest concentration of the analyte that a method can report with 99% confidence that the

measured concentration is distinguishable from method blank results. To determine the MDL during method development, seven drinking water samples were spiked at 0.100 ppb of TFA and processed through the entire method along with seven drinking water blanks. The standard deviation derived from the spiked sample recoveries was used to calculate the MDL using the following equation:

$MDL_S = (t)*(S)$
Where t is the Student single tailed t-test value for the 99% confidence level with n-1 degrees of freedom and S denotes the standard deviation obtained from n replicate analyses. For the n=7 replicates used to determine the MDL, t=3.143.

The MDL from the blanks (MDL_B) was set to the highest numerical result for a blank, based on the EPA's procedure for having some, but not all, method blanks giving numerical results. The results for the standard deviations and MDL are in Table 4.

13.3. The Lowest Concentration Minimum Reporting Level (LCMRL) fortification levels and calculated result are shown in Table 5. The LCMRL is 0.083 ppb. The calculations were performed using the EPA's RStudio LCMRL Calculator.

13.4. MRL passed the EPA confirmation criteria at 0.085 ppb. The results for this MRL confirmation are in Table 6.

13.5. Method Validation consisted of the analysis of reagent water spiked at five different levels (0.065, 0.130, 0.650, 1.50, and 10.0 ppb) and analyzed in five separate data sets on separate days. Recoveries for these validation samples are shown in Table 7.

13.5.1. Finding tap water with incurred TFA levels below 1/3 the MRL was difficult and thus was not included in the validation study. A variety of water matrices, both raw and treated, were spiked at low-, mid-, and high-levels to investigate interferences. While the low- and mid-levels recoveries were impacted by incurred TFA levels, the recoveries for the high-level spikes, and the RSDs for all levels were acceptable. See Table 8.

13.6. STORAGE STABILITY STUDY - A storage stability study was completed. The storage stability study consisted of three replicates spiked at 0.650ppb tested over a 28-day period. Amber HDPE

bottles containing tap water were spiked and stored in the refrigerator, and 1 mL aliquots of each were removed to be analyzed on days 0-28. A LRB and a LFB (0.500 ppb) were also prepared on each analysis day and analyzed with the storage stability samples. This storage stability study shows TFA stability through day 28. The results are shown in Table 9.

14. Pollution Prevention

14.1. For information about pollution prevention applicable to laboratory operations described in this method, consult: Less is Better, Guide to Minimizing Waste in Laboratories, a web-based resource available from the American Chemical Society at www.acs.org.

15. Waste Management

15.1. The Agency requires that laboratory waste management practices be consistent with all applicable rules and regulations, and that laboratories protect the air, water, and land by minimizing and controlling all releases from fume hoods and bench operations. In addition, compliance is required with any sewage discharge permits and regulations, particularly the hazardous waste identification rules and land disposal restrictions.

16. References:

16.1. US EPA. November 2019. *Method 533: Determination of Per- and Polyfluoroalkyl Substances in Drinking Water by Isotope Dilution Anion Exchange Solid Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry*.

16.2. US EPA. *Statistical Protocol for the Determination of the Single-Laboratory Lowest Concentration Minimum Reporting Level (LCMRL) and Validation of Laboratory Performance at or Below the Minimum Reporting Level (MRL)*; EPA 815-R-05-006; Office of Water: Cincinnati, OH, November 2004.

16.3. US EPA. *Technical Basis for the Lowest Concentration Minimum Reporting Level (LCMRL) Calculator*; EPA 815-R-11-001; Office of Water: Cincinnati, OH, December 2010.

16.4. US EPA. *Instructions for Determining LCMRLs using RStudio*, EPA ALS-6-0143; Office of Water: Cincinnati, OH, July 2024. https://github.com/USEPA/LCMRL_calculator

16.5. P. Ziese, et al. LC-MS/MS Analysis of Trifluoroacetate in Groundwater and Drinking Water using a bioZen® Glycan 2.6 µm 150 x 2.1 mm column (TN72660220_W). **2020**.

https://www.phenomenex.com/1a4774fb-e74c-433a-87ff-6b99efe7c96d (accessed February 2, 2023).

16.6. S.-H. Liang, J.A. Steimling, M. Chang. Analysis of ultrashort-chain and short-chain (C1 to C4) per- and polyfluorinated substances in potable and non-potable waters. *J. Chromatogr. Open* **2023**, *4*, Article 100098, 10.1016/j.jcoa.2023.100098

17. Tables, Figures, and Method Performance Data

Table 1. HPLC Method Conditions*

Time (min)	%Organic Phase	Flow Rate (mL/min)
Initial	85.0	0.550
7.0	85.0	0.550

*Phenomenex bioZen 2.6 µm Glycan LC column 150 x 2.1 mm. 50 µL injection into a 100 µL loop; run time 7 minutes.

Table 2. MS Method Conditions

MS Conditions for CAC (Sacramento, CA) AB Sciex 5500	
Polarity	Negative
Ion Spray Voltage	-4500V
Curtain Gas	Medium
Temperature	450°C
Ion Source Gas 1	40
Ion Source Gas 2	60

Table 3. Retention Times and MS/MS Method Conditions[a]

Analyte	RT (min)	Precursor Ion (m/z)[b]	Product Ion (m/z)[b]	Declustering Potential (V)	Collision Energy (V)[c]
TFA	3.27	**113**	**69**	-54	-11
$^{13}C_2$-TFA	3.27	115	70	-54	-12

[a.] Quantitation Precursor and Product Ions are in bold

[b.] Precursor and product ions listed in this table are nominal masses. During MS and MS/MS optimization, the analyst should determine precursor and product ion masses to one decimal place by locating the apex of the mass spectral peak (e.g., m/z 112.8→69.0 for TFA). These precursor and product ion masses (with at least one decimal place) should be used in the MS/MS method for all analyses.

[c.] Nitrogen used as collision gas.

Table 4. The Determination of Method Detection Limit (MDL) in Reagent Water Spiked at 0.100 ppb.

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9	SD	MDL
Spike	0.1118	0.1002	0.1052	0.0939	0.0953	0.1013	0.1006	0.0919	0.1052	0.006	0.018
Blank	ND	0.0131	ND	ND	ND	ND	ND	ND	ND	ND	0.013

EPA MDL definitions set the MDL to be the higher value of the MDL_S and MDL_B. Therefore, the TFA MDL=0.018 ppb.

Table 5. Lowest Concentration Minimum Reporting Limit for TFA

Analyte	Fortification levels, ng/mL	LCMRL
TFA	0, 0.065, 0.075, 0.100, 0.130, 0.650, 1.50, 10.0	0.083

Table 6. The Confirmation of Method Reporting Limit (MRL) in Reagent Water Spiked at 0.085 ppb.

Sample	Conc. (ppb)
1	0.1025
2	0.0877
3	0.0826
4	0.0812
5	0.0767
6	0.0975
7	0.0827
Mean	0.087
SD	0.009
Half Range	0.037
Upper Limit	146%
Lower Limit	59%

Lower Limit >50% and Upper Limit <150%
0.085 ppb PASSES as the MRL for TFA.

Table 7. Method Validation in Pre- and Post-treated Drinking Water

		Spike Level Recovery				
Water Source	Day	0.065 ppb	0.130 ppb	0.650 ppb	1.50 ppb	10.0 ppb
Reagent	1	108.92%	112.69%	100.15%	94.67%	101.75%
	2	152.62%	123.23%	87.37%	93.49%	101.94%
	3	83.54%	102.15%	87.00%	99.07%	96.76%
	4	93.08%	92.46%	94.25%	93.63%	98.99%
	5	106.77%	96.92%	98.29%	99.80%	98.78%

		Spike Level Recovery				
Water Source	Day	0.065 ppb	0.130 ppb	0.650 ppb	1.50 ppb	10.0 ppb
Tap*	1	N/A	N/A	108.7%	101.9%	102.4%
	2	N/A	N/A	107.5%	101.2%	104.4%
	3	N/A	N/A	107.8%	101.0%	101.8%
	4	N/A	N/A	115.9%	99.2%	103.8%
	5	N/A	N/A	123.7%	109.2%	107.9%

* The lowest two spike levels in tap water (0.065 and 0.130 ppb) are marked as N/A as the incurred levels of TFA in those samples are ≥1/3 the spike level.

Control Limits (reagent water)	
Mean	100.7%
SD	13.6%
RSD	13.5%
UCL	141.7%
LCL	59.8%

Table 8. Matrix Testing on Raw and Finished Drinking Water

		Blank		0.065 ppb		0.650 ppb		10.0 ppb	
		Average	%CV	Average*	%CV	Average*	%CV	Average	%CV
Sacramento River	Raw	0.344	2.1%	N/A	2.8%	N/A	4.6%	99%	1.8%
	Finished	0.337	2.2%	N/A	2.5%	N/A	2.8%	100%	3.6%
EA Fairbairn	Raw	0.314	0.2%	N/A	1.0%	N/A	3.0%	101%	0.5%
	Finished	0.309	3.7%	N/A	0.3%	N/A	0.3%	101%	3.2%

* The recoveries for the lower two spike levels (0.065 and 0.650 ppb) are marked as N/A as the incurred levels of TFA in those samples are ≥1/3 the spike level.

Table 9. Storage Stability Study in Tap Water
Spiked at 0.650 ppb.

Average Daily Recovery (n=3)						
Day 0	Day 2	Day 4	Day 7	Day 14	Day 21	Day 28
93.2%	87.4%	87.4%	88.5%	90.5%	82.1%	102.7%

Table 10. Initial Demonstration of Capability (IDC) QC Requirements

Method Reference	**Requirement**	**Specification**	**Acceptance Criteria**
9.1.1	Demonstration of precision and accuracy	Analyze 7 replicate Laboratory Fortified Blanks (LFBs) at the calibration midpoint.	Percent relative standard deviation <15%. Mean percent recovery >70% and <130%.
9.1.2	Demonstration of acceptable system background	Analyze 5 Laboratory Reagent Blanks (LRBs).	TFA concentration must be less than one-third the Minimum Reporting Level (MRL) in each LRB.
9.1.3	MRL confirmation	Fortify and analyze 7 replicate LFBs at the proposed MRL concentration. Confirm that the Upper Prediction Interval of Results (PIR) and Lower PIR meet the recovery criteria.	Upper PIR ≤150% Lower PIR ≥50%
9.1.4	Quality Control Sample (QCS)	Prepare a QCS near the center of the calibration with TFA from a source independent from the calibration standards.	Percent recovery >70% and <130% of the true value

Table 11. Analysis Batch QC Requirements

Method Reference	**Requirement**	**Specification and Frequency**	**Acceptance Criteria**
10	Initial Calibration	Use internal standard technique to generate a linear or quadratic calibration curve. Use at least 5 standard concentrations. Evaluate the calibration curve as in (10.4.3)	Analytes at or below the MRL must be within 50-150% of the true value. All other levels must be within 70-130% of the true value. RSE ≤ 15% (preferred) or $r^2 > 0.990$.
9.2.1	Laboratory Reagent Blank (LRB)	Analyze one LRB per Analysis Batch.	TFA concentration must be less than one-third the Minimum Reporting Level (MRL) in each LRB.
9.2.2	Continuing Calibration Check (CCC)	Calibration standards at MRL-, mid-, and high-level concentrations. One per Analysis Batch.	Percent recovery for MRL-level must be within 50-150% of the true value. All other levels must be within 70-130% of the true value.
9.2.3	Laboratory Fortified Blank (LFB)	Reagent water fortified at low-, mid-, and high-levels, rotating between batches. Analyze 1 per Analysis Batch.	Percent recovery for each LFB must be 70-130% of the true value (or 50-150% for concentrations ≤2xMRL)
9.2.5 / 9.2.6	Laboratory Fortified Sample Matrix (LFSM) and LFSM Duplicate or Field Duplicate (FD)	Fortify greater than native TFA concentration (if known). One set in Analysis Batches containing drinking water; two if 20 or more field samples. One set in Analysis Batches containing ambient water; two if 20 or more field samples.	Mean percent recovery of LFSM and LFSMD pair must be 70-130% of the true value (or 50-150% for concentrations ≤2xMRL) Relative percent difference (RPD) ≤30% (≤50% if analyte concentration ≤2xMRL). Qualify results for samples failing these limits as “suspect–matrix”.

Method Reference	**Requirement**	**Specification and Frequency**	**Acceptance Criteria**
9.2.7	Retention Time (RT)	The retention time for each field sample in an Analysis Batch must be ±0.05 min from the RT of the calibration standards of that batch.	Field Sample RT ± 0.05 min of the Initial Calibration RT
9.2.8	Quality Control Sample (QCS)	Assay 1 QCS for each new lot of calibration standards. Prepare the QCS near the center of calibration with TFA from a source independent of the calibration standards.	Percent recovery >70% and <130% of the true value