Technical Report

Multi-Laboratory Validation Study for Quantitation of Trifluoroacetate (TFA) by CAC-TFA-1.0

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1. Introduction:

1.1. Background

Trifluoroacetate (TFA) is an ultrashort-chain perfluoroalkyl substance (PFAS). It is the smallest of the perfluorinated carboxylates and considered fully mobile and highly persistent in the environment. TFA is stable in the environment and can persist in soil, water, and air for extended periods, likely to disrupt metabolic and endocrinal pathways and may cause developmental toxicity. TFA can form from the breakdown of a variety of chemicals, such as longer chain PFAS, pharmaceuticals, HFCs, and fire-fighting foams. Levels of TFA, and other ultra-short chain (USC) PFAS, are trending higher in aquatic environment and drinking waters due to their persistence. It has become more important to measure and monitor USC PFAS to assess the potential risks. The State Water Resources Control Board, Division of Drinking Water (DDW) has contracted CDFA's Center for Analytical Chemistry to develop a method for use in monitoring this compound in drinking water.

1.2. Method Summary

The analytical method for this study was validated and the single laboratory validation results are summarized in the following section. Refinements were made to that method based on the comments and results from the four participating laboratories in the multilaboratory validation study (MLVS). Those updates are released as CAC-TFA-1.1. This complete method is attached to this report as Appendix A.

The analytical method includes sample preparation and sample analysis procedures for raw and finished ground- and surface-sourced drinking water. The limited sample preparation includes spiking an isotopically labeled internal standard. The samples are directly injected into a liquid chromatography tandem mass spectrometer (LC/MS/MS) system for quantitative analysis.

1.3. Summary of the Single-Laboratory Study

The single-laboratory validation was performed by CDFA's Center for Analytical Chemistry Research and Development Unit (Sacramento, CA) (CAC-R&D), the laboratory originally contracted by State Water Resources Control Board, Division of Drinking Water (DDW) to develop a laboratory Standard Operating Procedure (SOP) for the analysis of TFA.

The single-laboratory validation study was intended to generate method performance data for the necessary aqueous matrices (surface and groundwater, raw and finished). Due to the ubiquitous nature of TFA, many of the near-MRL level spikes have exaggerated recoveries, sometimes over 500%. However, any of the test matrices that were spiked at least three times higher in concentration than their incurred level had acceptable recoveries between 70-130% when spiked greater than twice the MRL, and for the small number of samples that had very low levels of incurred TFA, MRL-level

spikes had recoveries between 50-150%. From these results it was decided to only include water sources with very low levels of background TFA in the MLVS.

The required instrumentation for this method has become commonplace in many full-service environmental laboratories. This availability, along with limited sample preparation, indicates that this method should be accessible, and possible to be implemented in a typical full-service environmental laboratory.

2. Study Management, Objectives, Design, and Implementation:

While this MLVS is not designed to support an alternate test procedure (ATP) application, the number of matrices and statistical analyses of the data reflect what would be required for an ATP for national use.

2.1. Study Management

Four laboratories (three commercial contract laboratories and one municipal water district laboratory) volunteered to participate in the study, under the authorization and stewardship from DDW. The four laboratories are listed in Table 2-1. All four laboratories contributed to the analysis of the aqueous matrices in this report (raw groundwater and finished groundwater-sourced drinking water). For the purposes of this study, the laboratories were randomly assigned numbers, which were used to maintain the anonymity of the results, these numbers do not correspond to their order of appearance in Table 2-1.

The CAC Quality Assurance unit (CAC-QA) oversaw the spiking of matrix and Proficiency Testing (PT) samples, and managed the shipment and received all data packages from the laboratories. Analytical standards were provided to the participating laboratories by CAC. Participating labs were also connected with a representative of Phenomenex Inc. who offered to provide LC columns for this study, if there was such a need. CAC-QA, with an established operational set-up and expertise to conduct PT schemes, prepared the PT samples shipped as part of this study, while CAC-R&D prepared the aqueous matrix samples.

CAC served as the method consultant to the MLVS and was available to clarify any questions or concerns.

Table 2-1. Participating Laboratories

Laboratory	Location	Role
Participating MLVS Labs		
Babcock Laboratories, Inc.	Riverside, CA	MLVS participant
Orange County Water District	Fountain Valley, CA	MLVS participant
Eurofins Environment Testing	Sacramento, CA	MLVS participant
McCampbell Analytical	Pittsburg, CA	MLVS participant
Ancillary Labs		
CDFA Center for Analytical Chemistry – Research and Development	Sacramento, CA	Single laboratory validation, preparation of matrix samples
CDFA Center for Analytical Chemistry – Quality Assurance	Sacramento, CA	Preparation of PT samples, evaluation of data

2.2. Study Objective and Design

The focus of the MLVS is to generate the necessary data to document the precision and accuracy and overall performance of the analytical method for quantitation of TFA in aqueous matrices. The primary objectives of this MLVS are to:

- Obtain data from aqueous matrices that are representative of the method's intended use.
- Obtain data from laboratories that are representative of those likely to use the method, but that were not directly involved in its development.
- Obtain feedback from laboratory users on the specifics of the method SOP.
- Use study data to evaluate the performance of the method.
- Develop QC acceptance criteria – average recovery and relative standard deviation – that will reflect method performance capabilities in real-world situations.

A brief description of the key points of this study design include:

- At least three laboratories, one of which is a municipal water laboratory.
- Two aqueous matrix samples from source and finished groundwater.
- Initial calibration of TFA by each laboratory.
- Initial Demonstration of Capability (IDOC) by each laboratory.
- Analyses of PT sample and matrix spike samples from each aqueous matrix.

This MLVS was conducted in two phases. The IDOC, which includes the initial calibration and verification of MRL level, and the method evaluation in the chosen aqueous matrices.

2.3. Matrix and Sample Selection

Two groundwater samples (one raw and one finished drinking water) were used for this phase of the MLVS. These were chosen to be representative of the expected real-world matrices analyzed by this method. These samples were collected from the Citrus Heights Water Treatment Plant. This location was chosen as it had previously been found to have very low incurred TFA levels, allowing it to be used for even the MRL-level spikes.

The MLVS was designed to include, for each water matrix analyzed, an 'unspiked' (native) sample, two replicates spiked at low concentration, and two replicates spiked at a mid-level concentration.

2.4. Selection of Spiking Levels and Aqueous Media

The sample matrices were collected by DDW. The chosen sample matrices were raw and finished water from the Citrus Heights Treatment Plant. The TFA spiking levels were chosen by CAC to be at the proposed MRL for the low-spike, and below 10 ppb for

the mid-spike. CAC-QA separately selected the PT spike level so that it would be blind to everyone participating, including all other CAC units.

2.5. Preparation of Study Samples

Aliquots of groundwater for each of the raw and finished sources were prepared as follows: The sample bottles delivered by DDW were allowed to come to room temperature and then inverted several times to ensure homogeneity. A 10mL matrix sample was prepared in a 30 mL amber leakproof HDPE bottle for each level. The water samples prepared and shipped by CAC were, for each matrix: one unspiked sample, duplicates at the MRL-spike level, and duplicates at the mid-spike level. After spiking, they were mixed well, sealed and stored in a refrigerator until they were packaged and shipped to the participating laboratories. The samples were shipped in ThermoSafe® insulated boxes with sufficient blue-ice packs to keep them cool, and a TempDot® sensor to ensure the samples stayed cold during transit.

As part of the MLVS, Proficiency Testing (PT) samples were also sent to the laboratories. An unknown spiked sample prepared by CAC-QA utilized reagent water as the matrix and shipped along with the study sample spikes. CAC-R&D analysts involved with the TFA method development had no participation in preparing these samples. CAC-QA also provided a PT sample to the CAC-R&D lab for analysis.

2.6. Storage Stability Study

During method development, a storage stability study was conducted to determine the hold time of TFA samples in finished tap water over the course of 28 days. The matrix samples were spiked in triplicate for this study, and triplicate tap water blanks were also stored at the same time. This allowed for the analysis of both the stability of the spiked TFA, but also the stability of any TFA concentration incurred. The data can be found in the method SOP in Appendix A, and the hold time was determined to be 28 days. The samples were stable through the end of the storage stability study.

3. Data Management, Data Validation, and Data Rules for Statistical Analysis:

3.1. Programmatic Overview

The data management process involved documented and approved instructions, meetings, consultation and communication when required, and review of laboratory packages. A “kickoff” meeting over Teams was scheduled to explain the expectations for the MLVS to the participating laboratories. Laboratories were provided with the method prior to the meeting in preparation and given the opportunity to review and get any questions or concerns clarified. They were also encouraged to ask questions via email or to schedule a one-on-one meeting throughout the MLVS as necessary. A written document containing the MLVS guidelines and passing QC criteria was also provided to the laboratories along with a data reporting template Excel workbook, to ensure uniform reporting.

3.2. Data Management

All result reporting forms, and raw data were submitted to CAC-QA via email. The labs were provided with an Excel workbook containing a sample handling sheet to document sample preparation and a template to report their analytical batch data and compute the IDOC results. The Excel template cells with embedded formulas were locked to prevent accidental corruption or unintended changes. The laboratories also submitted their instrument data such as chromatograms and transitions.

3.3. Data Validation

All data packages were reviewed by CAC-QA for completeness, compliance with the MLVS guidelines, and performance according to the QC metrics, see QC acceptance criteria in the SOP in Appendix A.

4. Calibration and Quantification:

Aqueous samples were analyzed by LC/MS/MS in MRM mode. The mass spectrometer was calibrated for masses to ensure the accuracy of the mass to charge ratio (m/z) values assigned to the instrument per the manufacturer's instructions. After the mass calibration had been verified, quantitative standards were used for a minimum five-point calibration for TFA.

4.1. Mass Calibration and Mass Calibration Verification

Each laboratory performed mass calibration and verification in accordance with their respective instrument manufacturer's instructions. The laboratories were instructed to use the same precursor and product ion masses (113/69), as TFA is very small and only has one reliable transition.

4.2. Initial Calibration

To provide each laboratory with the target analyte, CAC procured standards from Cabridge Isotope Laboratories, Inc. (CIL) and HPC Standards Inc, two commercial standards vendors. By providing the standards to all laboratories, the study variability that would have resulted from having each laboratory prepare standards was reduced. This also reduced the direct costs to each laboratory for their participation. The standards provided by CAC were used by the laboratories to create all calibration standards, calibration verification, and spiking solutions used in the MLVS. CAC-R&D and CAC-QA also used the same lot of standards to prepare the matrix samples and PT samples. In addition, CAC provided the laboratories an internal standard, procured from CIL.

Each laboratory calibrated their LC-MS/MS instrument using a series of calibration standards similar to those described in the method SOP (Appendix A); five to eight calibration levels between 0.040 ppb and 20.0 ppb. Each laboratory chose a different distribution of calibration levels. A minimum of five calibration standards was required for a valid analysis with the lowest calibration standard being at or below the MRL. The laboratories were allowed to use a linear or quadratic regression using peak areas and the internal standard technique. The SOP outlines calibration and quantification using an internal standard where TFA's response is compared to the isotopically labeled $^{13}C_2$-TFA.

Analytes at or below the MRL are required to be within ±50% of the true value, all other levels, within ±30% of the true value. Regression coefficients, r or r^2, were required to pass the following criteria: $r>0.995$, $r^2>0.990$. The relative standard error of the calibration curve needed to be less than or equal to 15%. An initial calibration was required to be submitted by each laboratory as part of the IDOC prior to receiving spikes/PT samples.

4.3. Calibration Verification and Instrument Sensitivity Check

Each laboratory analyzed multiple Continuing Calibration Check (CCC) samples in each batch to verify the integrity of the curve throughout the batch. The batch starts with a standard at the method MRL, followed by another CCC after every 10 samples, alternating between mid- and high-level calibration standards. MRL-level CCCs were required to recover within ±50% of their true value, mid- and high-level CCCs were required to recover within ±30% of their true value. No sample results were eliminated from the study due to CCC failures.

5. Initial Demonstration of Capabilities:

In addition to performing the initial calibration, laboratories submitted the results for IDOC. The IDOC included a precision and accuracy analysis, system background check, and MRL confirmation. In addition to those metrics discussed below, each laboratory also demonstrated satisfactory results for the chromatographic requirements (peak shape, change in retention time (RT), and internal standard area deviation from the calibration).

5.1. Demonstration of Precision and Accuracy (P&A) Results

Laboratories were required to spike seven replicate Laboratory Fortified Blanks (LFBs) fortified near the center of their calibration range. These LFBs were prepared and analyzed in the same manner as study samples, per the method. A percent relative standard deviation of less than 15% and a mean percent recovery of ±30% of the true value were the required criteria for this demonstration. All laboratories met these criteria.

5.2. Acceptable System Background

Five Laboratory Reagent Blanks (LRB) were run in the same batch as the LFBs for the P&A. The laboratories were required to have a background TFA level of less than one-third of their MRL. All laboratories satisfied this requirement, however Laboratory 4 originally only analyzed two LRBs and was directed to run the full five LRBs with their matrix samples as required by the study design.

5.3. Minimum Reporting Limit Verification Analyses

The Minimum Reporting Limit for TFA using this method was set by the method development (MD) laboratory at 0.085 ppb. The participating laboratories were required to confirm their MRLs using the MD level as an initial reference point. If the MRL confirmation fails initially, the labs were instructed to increase the concentration until the MRL confirmation passed. Seven LFBs were required to be spiked at the proposed MRL for confirmation using the formulas in the method.

Evaluation of the MRL data indicates that two laboratories confirmed the MRL at 0.085 ppb, one lab confirmed 0.075 ppb as the MRL, and the remaining lab at 0.250 ppb.

5.4. Quality Control Sample (QCS)

A QCS prepared from a standard from a different source to that of the calibration standards was also required as part of the IDOC. Each laboratory was sent standards from multiple vendors and allowed to choose which they would use as their primary standard and which would be their QCS. The QCS was prepared by the labs as a mid-level spike was used to confirm the accuracy of the calibration standards. The QCS must be within ±30% of the true value. All laboratories had passing QCS values indicating accurate calibration curves for this MLVS. Laboratory 1 spiked at 0.100 ppb,

Laboratories 2 and 3 spiked at 0.500 ppb, and Laboratory 4 spiked at 1.00 ppb, the recoveries are shown in Table 5-1.

Table 5-1. IDOC Results

Laboratory	Precision (%RSD)	Accuracy (Mean %)	System Background (Mean ppb)	MRL (ppb)	UPIR	LPIR	QCS
1	1.4%	94.7%	0.000	0.085	114.1%	68.8%	93.0%
2	3.0%	92.4%	0.000	0.075	146.1%	79.8%	90.5%
3	14.5%	97.9%	0.000	0.085	140.9%	71.7%	116.2%
4	3.7%	99.2%	0.000	0.250	145.0%	86.8%	106.8%

6. Water Matrix Results:

Each participating laboratory was sent ten matrix samples from the Citrus Heights Water Treatment Plant, five prepared from raw groundwater and five from finished groundwater. In addition to these ten samples, each lab was also sent a field reagent blank to analyze. The Citrus Heights Water Treatment Plant was chosen as the source of the matrix water for the current MLVS due to prior testing of its water by CAC-R&D showing it to have little native TFA concentration, and demonstrated not to interfere in the analysis. This was essential so that the laboratories would demonstrate their ability to analyze samples at their MRLs, given the ubiquitous nature of TFA, no other drinking water source among those tested during method development was evaluated to be without a native concentration of TFA.

6.1. TFA Concentrations in Unspiked Matrices

Each laboratory received one 10 mL sample of each unspiked matrix, raw and finished ground-sourced drinking water. All laboratories reported native concentrations of TFA well below one-third of their MRLs. These levels are included in Table 6-1.

6.2. Matrix Spike Results

The spike recovery data from all laboratories is also shown in Table 6-1. The raw and finished water matrices were spiked in duplicate at the proposed MRL-level (0.085 ppb) and at a mid-level (5.00 ppb).

The results were mixed for the MRL-level samples. Laboratory 3 had some samples with internal standard areas that deviated from the expected ±40% from the calibration set and had ND results for the MRL-level matrix samples. The MRL-level spikes were below Laboratory 4's confirmed MRL level of 0.250 ppb and are reported as estimates. Laboratory 1 had a single MRL-level sample (in raw groundwater) fall below the passing QC criterion of 50% recovery, but all their other MRL-level samples passed.

The results from all laboratories at the mid-level had recoveries ranging from 59.8%-122.6% recovery. Laboratory 3 had mid-level recoveries below the passing QC criterion 70% recovery.

6.3. PT Results

Each laboratory was sent a PT sample prepared by CAC-QA for analysis. PT sample ensemble with matrices were packaged in an insulated shipping box (such as ThermoSafe®) with ice packs ensuring standard integrity. All PT samples were shipped together to ensure process integrity and uniformity. Participating labs were given four weeks of time with results required to be sent to the CAC - QA. Table 6-2 denotes the results.

Table 6-1. Unspiked and Spiked Matrix Results

	Raw (ppb)					Finished (ppb)					
Laboratory	Unspiked	MRL Rep. 1	MRL Rep. 2	Mid-Level Rep. 1	Mid-Level Rep. 2	Unspiked	MRL Rep. 1	MRL Rep. 2	Mid-Level Rep. 1	Mid-Level Rep. 2	Field Blank
1	ND	0.059	0.040	3.676	3.775	ND	0.046	0.057	3.787	3.857	0.001
2	ND	0.072	0.052	6.129	5.810	ND	0.070	0.064	5.845	6.101	0.000
3	0.000[a]	0.000[a]	0.000[a]	3.197	3.061	0.000[a]	0.000[a]	0.000[a]	3.167	2.990	0.000
4	ND	0.034[b]	0.077[b]	5.474	5.035	ND	0.081[b]	0.074[b]	5.465	5.385	ND
CAC-R&D	ND	0.054	0.060	4.685	4.877	ND	0.056	0.054	4.774	4.941	ND

[a] Laboratory 3 had internal standard areas >140% of their calibration areas for some samples, resulting in the MRL level samples reporting as ND.

[b] MRL level spikes for laboratory 4 were below the confirmed MRL and are reported here as estimates.

Table 6-2. PT Results

Laboratory number	Target Value (ppb)	Recover Conc. (ppb)	Percent Recovery
1	2.700	2.319	85.9%
2	2.700	3.719	137.7%
3	2.700	0.818	30.3%
4	2.700	3.124	115.7%
CAC-R&D	2.700	2.912	107.9%

7. Summary:

7.1. Preparatory Batch QC

Per the CAC-TFA-1.0 method, an analysis batch consists of up to 20 field samples, a method blank, a continuing calibration check, laboratory fortified blank, and a laboratory fortified sample matrix and laboratory fortified sample matrix duplicate (or field duplicate) pair. A batch should begin, and end, with a continuing calibration check sample.

7.1.1. Method Blank

Method blanks, referred to as Laboratory Reagent Blanks (LRB) in this method, are included in the method to evaluate the potential for background contamination to be introduced during sample preparation in the laboratory. The TFA concentration in the LRB must be less than one-third the MRL. This requirement was met by all participating laboratories during the matrix and PT sample batches.

7.1.2. Ongoing Precision and Accuracy Analyses

Ongoing Precision and Accuracy samples, represented in this method by the batch LFB requirements, are included to evaluate the efficiency of the sample preparation processes over time. A LFB prepared in the same manner as the study samples is included in every preparation batch. CCC samples at mid- and high-levels are distributed throughout each batch as a check on calibration as well as ongoing accuracy. These CCCs were included in all laboratories' batches

This method also calls for the inclusion of Laboratory Fortified Sample Matrix (LFSM) and Laboratory Fortified Sample Matrix Duplicate (LFSMD), or a set of Field Duplicates (FD). LFSM and LFSMD are field samples, spiked with a known amount of TFA. FD are duplicate samples, collected at the same time in the field and treated identically through all sampling and laboratory procedures. These are included to check for any contamination from sample collection, preservation, storage and laboratory procedures in the samples of each batch. While not all labs included these samples during the MLVS, they should be included during routine use of this method.

7.1.3. Low-Level Ongoing Precision and Accuracy Analyses

Low-Level Ongoing Precision and Accuracy samples, the lowest-level CCC samples in the method, are included in each in the first analysis batch each day to evaluate method performance at the at the MRL. All laboratories except Laboratory 1 included a low-CCC in their matrix and PT sample batches.

7.2. Matrix Spike Analyses

Ongoing QC criteria of ±30% recovery when spiking ≥2*MRL, and ±50% recovery when spiking <2*MRL, (Appendix A, Section 9.2.4) was applied to evaluate the results. Excluding Laboratory 3, the mid-level spike results in both matrices were satisfactory,

and the MRL-level spike results were satisfactory for labs whose MRLs were lower than the spiking level (Laboratory 4's MRL was above the spiking level).

7.3. PT Result Analyses

All participating laboratories, including the CAC-R&D lab, performed the PT sample analysis satisfactorily, with the exception of Laboratory 3, based on the target amount and applying a PT requirement of the recoveries to be within ±40%. The overall recovery is 111.8 % and the RSD is evaluated to 19.1%. This calculation does not include Laboratory 3.

Although the ongoing QC criteria is set at ±30%, using a ±40% threshold for PT evaluation is realistic given the data variability, as indicated by the RSD of 19.1%. The elevated RSD suggests a moderate level of dispersion with the PT performance results. This dispersion can be seen as representative of the potential variability between labs based on the choices each laboratory made to optimize their chromatographic conditions.

8. Conclusions:

The objectives of this MLVS were achieved: validation of the CAC-TFA-1.0 method and the determination that the method can be implemented at a typical mid-sized full-service environmental laboratory. Overall, the data from the MLVS demonstrates that CAC-TFA-1.0, as written, is robust and is suitable for laboratories with similar instruments of different manufacturers and models. Points of additional clarity have been added to the SOP as version CAC-TFA-1.1. Specifically, it has been noted that the LFSM/LFSMD should be spiked greater than the native concentration of TFA instead of just at the mid-level of the calibration. Some corrections have also been made to Tables 10 and 11 in Section 17 to more accurately reflect the QC requirements listed in Section 9.

The results of the participating labs in this study have met the requirements stated in the method for:

- Mass Calibration and Verification
- Initial Calibration
- Calibration verification and sensitivity check
- Initial Precision and Accuracy
- Confirmation of MRL
- Batch QC samples

The suitability of the method to detect and quantify TFA in ground-sourced drinking water (both raw and finished) was successfully demonstrated through the analysis of spiked samples of those real-world matrix types. This suitability should also extend to raw and finished surface-sourced drinking water as well, though a surface source with a low enough native concentration of TFA to allow for MRL-level testing was unable to be found for this study. Method blank results demonstrated no bias from background contamination during sample preparation, though this remains a concern due to the increasing ubiquity of TFA. The Initial and Ongoing Recoveries and the Low-CV recoveries demonstrated that the QC acceptance criteria in the method were satisfactory for inclusion in the finalized method.

Finding a suitable background water matrix free of any incurred TFA could be very challenging and may require several pre-evaluations to ensure appropriate blank matrix material for this analysis. We encourage studies making use of this method to require the collection of Field Duplicate (FD) pairs, instead of LFSM/LFSMD pairs, to avoid needing to pre-analyze the field matrix to determine an appropriate spiking level. Instead ensure that the relative percent difference (RPD) between the FD pair meets the method QC requirements (Appendix A, Section 9.2.6).

9. References:

9.1. 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.

9.2. 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.

9.3. 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.

9.4. 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

9.5. 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).

9.6. 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

Appendix A: Standard Operating Procedure for CAC-TFA-1.1

[Changes from CAC-TFA-1.0 to CAC-TFA-1.1 have been underlined.]

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

Version 1.1
June 19, 2025

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This method was prepared under the contract with the State Water Resources Control Board, Division of Drinking Water 22-007-400-1.

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 10^{th} 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

Appendix B: Guidelines for Interlaboratory Validation of Method CAC-TFA-1.0

Guidelines for Interlaboratory Validation of Method CAC-STX-1.0

This set of guidelines is written for the purpose of guiding the labs who volunteered to participate in in the interlaboratory validation of method CAC-STX-1.0: Quantitation of Total (Extracellular and Intracellular) Saxitoxin by Enzyme-linked Immunosorbent Assay (ELISA).

1.1. Initial Demonstration of Capability (IDOC)

1.1.1. Labs will be provided with the SOP and sent ELISA kits via overnight shipping.

1.1.1.1. Verify the integrity of the kits upon receipt.

1.1.1.1.1. Inspect package for damage

1.1.1.1.2. Confirm all components are present.

1.1.1.1.3. Verify expiration dates.

1.1.1.2. Store kits and standards according to label conditions.

1.1.1.3. Follow the SOP to perform the Initial Demonstration of Capability (IDOC)

1.1.1.4. Note: all dilutions of standards need to be done with 1X diluent buffer (prepared from 10X diluent buffer provided in kits).

1.1.2. IDOC Data submission and competency verification. Submit IDOC data to ensure competency and address any procedural challenges before Water Matrix / PT sample distribution.

1.1.2.1. Table 1: QC requirements summary.

Method Reference	**Requirement**	**Specification**	**Acceptance Criteria**
9.1.1	Demonstration of precision and accuracy	Analyze 7 replicate Laboratory Fortified Blanks (LFBs) at 0.07 ng/mL.	Percent relative standard deviation (RSD) <15%. Mean percent recovery >70% and <130%.
9.1.2	Demonstration of acceptable system background	Analyze 5 Laboratory Reagent Blanks (LRBs) distributed throughout a plate.	STX concentration must be less than one-half the Minimum Reporting Level (MRL) in each LRB. STX < 0.5 x MRL

Method Reference	Requirement	Specification	Acceptance Criteria
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 STX from a source independent from the calibration standards.	Percent recovery >70% and <130% of the true value

1.1.2.2. The initial proposed MRL should be 0.03 ppb based on the method development results.

1.1.2.2.1. MRL confirmation spikes can be made by diluting the kit calibration standards in 1X diluent buffer.

1.1.2.2.2. *For example*: To achieve a 0.03 ppb spikes, dilute 75µL of Standard 5 (0.4ppb) to 1mL in 1X diluent buffer.

1.2. Method Evaluation in Surface-source Water

1.2.1. Labs will be sent 2 sample waters, one each from a treated surface-source drinking water and a raw surface-source water. 5 aliquots need to be taken from each water. These aliquots will be used to prepare a single unspiked sample, and duplicate low- and duplicate mid-spiked samples for each water source.

1.2.1.1. Verify the receipt of these samples as in 1.1.1.1 with the following addition:

1.2.1.1.1. Verify TempDot has not gone out of range during shipping.

1.2.2. A Sample Handling sheet will be provided in the Report Template workbook, please fill it out for sample traceability.

1.2.3. Working standards for spiking these samples will be prepared and provided by the CDFA/CAC QA unit.

1.2.3.1. Spike the sample aliquots provided according to Table 2

Sample	Volume Standard (µL)	Volume Sample (µL)
Unspiked x1	0	1000
Low-spike x2 (0.03 ppb)	30.0	970
Mid-spike x2 (0.10 ppb)	100	900

1.2.4. Immediately prepare 1mL samples by mixing 100 µL of 10X diluent buffer with 900 µL of the spiked samples. This will result in a dilution factor of 1.1.

1.2.5. Unspiked samples should be run in a separate batch to confirm absence of incurred saxitoxin prior to running spiked samples.

1.3. Proficiency Testing

1.3.1. After acceptance of IDOC data, each lab is sent one blind PT standard

1.3.1.1. Lab is required to spike the PT standard into reagent AND local tap water as follows:

1.3.1.1.1. Aliquot 100 µL of PT working standard and dilute to 1 mL in reagent/tap water.

1.3.1.1.2. Immediately prepare each 1mL sample by mixing 100 µL of 10X diluent buffer with 900 µL of the spiked reagent/tap water. Tap water samples should also include 0.1mg/mL of ascorbic acid to quench residual chlorine.

1.3.1.1.2.1. Ascorbic acid should be added as a neat compound, to not affect the final volume of the samples.

1.3.2. Submit all data to CDFA/CAC QA unit.

1.3.2.1. CDFA QA Supervisor, Sarva Balachandra, sarvamangala.gunjur@cdfa.ca.gov

1.3.2.2. Use provided Report Template workbook to report results for IDOC, water samples, and PT

1.3.2.2.1. Additional Batch Data Worksheets may be duplicated from the template for each batch.

1.3.2.3. In addition to the resulting data, please also submit:

1.3.2.3.1. Raw absorbance data

1.3.2.3.2. Microtiter plate legend

Appendix C: Data Reporting Template for MLVS of Method CAC-TFA-1.0

Sample Handling Sheet

Method # : CAC-STX-1.0
Analysis:
Matrix:

CDFA SampleID	LAB SampleID	prepared by/date:	Matrix ID	sample volume (mL)	spk soln. ID	Volume spiked (mL)	Final Volume (mL)		comments

QC Samples

QC SampleID	LAB SampleID	prepared by/date:	Matrix ID	sample volume (mL)	spk soln. ID	Volume spiked (mL)	Final Volume (mL)		comments
LRB			Reagent		n/a		1.00		
LFB			Reagent						

Reagent _____ _____ _____
Water _____ _____ Pipette ID _____

Approved By: Mohammad Chehelamirani Rev. 6 12/18/2024

Batch: _____
Kit Vendor: _____
Kit Lot#: _____

Please also attach raw absorbance data and plate map legend

Date Analyzed	SampleID	Spiked Conc. (ppb)	Calculated Conc. (ppb)	Recovery	%CV
				N/A	
				N/A	
				N/A	
				N/A	
				N/A	
				N/A	
				N/A	
				N/A	
				N/A	
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				N/A	
				N/A	
				N/A	
				N/A	
				N/A	
				N/A	
				N/A	

4PL Parameters	Value
A	
B	
C	
D	
R^2	