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Development and Preliminary Evaluation of an LC-MS/MS Method for Trifluoroacetic Acid for Future Application to Surface Water

THESIS

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MASTER OF SCIENCE

in Environmental Engineering

by

Hui-Chung Hsu

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DEDICATION

To

my parents who believed in me when others did not

my partner, children, and therapist who helped me through some of the toughest times

my professors who shared their support, time, and knowledge selflessly

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ABSTRACT OF THE THESIS

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Assistant Professor Christopher Olivares, Chair

Trifluoroacetic acid (TFA) is an ultrashort-chain per- and polyfluoroalkyl substance (PFAS) that is small in molecular size, with strong acidity, high water solubility, and low hydrophobicity. These properties contribute to high environmental mobility and substantial analytical challenges. Other than direct environmental release, TFA also serves as the degradation endpoint for various anthropogenic precursors, such as fluorinated refrigerants, pesticides, pharmaceuticals, and industrial chemicals. Conventional reversed-phase chromatography PFAS methods provide insufficient TFA retention because its small size, high polarity, negative charge, and low hydrophobicity limit interaction with C18 phases. This poor retention can cause early elution with matrix constituents and greater sensitivity to background contamination from various laboratory materials such as storage tubing and instrumentation used during dilution. Hydrophilic column chemistries, such as hydrophilic interaction liquid chromatography (HILIC), can improve the retention of highly polar compounds like TFA and help overcome limitations associated with conventional reversed-phase PFAS separations. HILIC columns overcome some of these limitations by providing greater retention of highly polar analytes through partitioning into a water-

rich layer on the polar stationary phase, along with additional polar and ionic interactions that can improve separation from early-eluting matrix components.

This study developed and evaluated a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for determining TFA, using laboratory-prepared aqueous samples as a foundation for future application to environmental surface water. The analysis was performed on an Agilent 6470 triple-quadrupole mass spectrometer operated in negative electrospray ionization mode with an Agilent AdvanceBio HILIC column. Method development evaluated chromatographic retention, mobile-phase conditions, injection parameters, multiple-reaction-monitoring conditions, dwell time, calibration strategy, and control of laboratory background. The final TFA acquisition method monitored the transition m/z 113 → 69 with a 100 ms dwell time, and TFA eluted at approximately 2.20 min. Calibration standards from 75 to 300 ng/L produced a concentration-dependent response, with a 1/x-weighted linear calibration yielding an R^2 of 0.9972. Blank responses were more than two orders of magnitude lower than the lowest calibration standard. Preliminary calculations based on calibration slope and blank variability produced estimated detection and quantitation limits of approximately 0.42 and 1.27 ng/L. However, this was not confirmed with low-concentration validation samples.

The results show the feasibility of the developed HILIC LC-MS/MS approach for TFA measurement under controlled laboratory conditions. Formal assessment of accuracy, precision, low-level quantitative performance, matrix effects, and performance in surface water remains crucial before the method can be considered validated for environmental monitoring.

CHAPTER 1: INTRODUCTION

1.1 PFAS and the Emergence of Ultrashort-Chain Compounds

Per- and polyfluoroalkyl substances (PFAS) are a broad class of synthetic fluorinated compounds used in industrial processes and consumer products because of their thermal stability, chemical resistance, low surface energy, and surfactant behavior.[1] These properties have supported applications in firefighting foams, textiles, paper and food-contact coatings, cleaning agents, electronics, pharmaceuticals, pesticides, and fluoropolymer production.[1] The same carbon-fluorine chemistry that makes many PFAS commercially useful also leads to their persistence and widespread distribution in air, water, soil, sediment, wildlife, food, and humans.[1,2]

PFAS research and regulation initially focused on long-chain perfluoroalkyl acids such as perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) because of their persistence, bioaccumulation, and documented toxicological effects.[3,4] Restrictions on several long-chain compounds have encouraged the use of shorter-chain alternatives and fluorinated precursors.[1,4] Short-chain and ultrashort-chain products are generally less hydrophobic. They may bioaccumulate less in aquatic organisms, but their high mobility, poor removal by conventional treatment, continual emissions, and potential for irreversible accumulation create a different form of long-term concern.[4,5]

The terminology used for ultrashort-chain PFAS is not completely uniform. In analytical literature, compounds containing one to three perfluorinated carbons are commonly described as ultrashort-chain PFAS. This group includes trifluoroacetic acid (TFA), with one perfluorinated carbon. Their molecular size and ionic character distinguish them from the longer-chain PFAS addressed by most standardized reversed-phase LC-MS/MS methods.[5]

1.2 Chemical Structure and Properties of TFA

a. b.

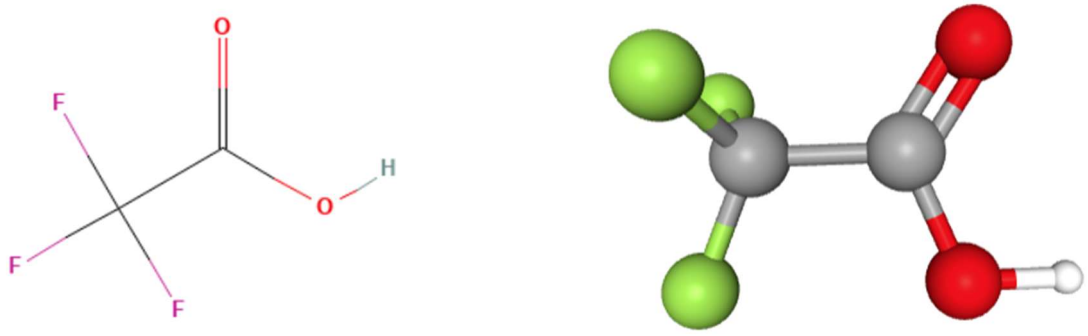

Figure 1 TFA structure (a. 2D; b. 3D)
National Center for Biotechnology Information (2026). PubChem Compound Summary for CID 6422, Trifluoroacetic acid. Retrieved July 29, 2026, from https://pubchem.ncbi.nlm.nih.gov/compound/Trifluoroacetic-acid.

TFA has the molecular formula CF_3COOH and consists of a fully fluorinated methyl group attached to a carboxylic acid. It is a strong organic acid with an experimentally determined pKa of approximately 0.03 ± 0.08.[6] It is present in environmental waters almost entirely as the trifluoroacetate anion (CF_3COO-). Its low molecular weight, strong acidity, high aqueous solubility, negligible hydrophobic partitioning, and resistance to biological and abiotic degradation allow it to move readily through the atmosphere, soils, surface waters, groundwater, and treatment systems.[5,7]

TFA is sometimes described as naturally occurring because it has been measured in old ocean water and other pre-industrial samples.[8] A critical evaluation of these data found substantial uncertainty in older measurements and no demonstrated environmental mechanism capable of explaining natural TFA formation at the reported scale. [8] As a result, claims of natural TFA sources should be treated with caution. The existing data are not strong enough to establish a significant

natural source, whereas many anthropogenic sources and precursor compounds are well documented.[8,9]

Unlike many long-chain PFAS, TFA generally shows a low tendency to bioaccumulate in aquatic organisms when evaluated using conventional measures like bioconcentration and bioaccumulation factors.[5] Due to its high water solubility, ionic character, and low hydrophobicity, it limits retention in fatty tissues and promotes its elimination from organisms.[5] Nevertheless, TFA's extreme persistence, continuous environmental input, tendency to accumulate in water, and efficient uptake by plants can result in widespread and prolonged exposure.[5,10]

1.3 Sources and Environmental Pathways

TFA enters the environment through direct emissions and the transformation of fluorinated precursors.[7,9,10] Direct sources include facilities that manufacture or use TFA and related fluorochemicals.[7,9] Secondary sources include atmospheric and environmental degradation of fluorinated refrigerants and blowing agents, pesticides, pharmaceuticals, and other compounds containing carbon-bound trifluoromethyl groups.[7,9,10] Wastewater treatment plants, landfills, industrial discharges, pesticide use, and thermal or destructive treatment of fluorinated materials can therefore contribute to TFA loading.[9,10]

Atmospheric precursor photooxidation can produce TFA, which enters the watershed via rain or dry deposition.[7,9] Agricultural precursor use can produce TFA in soils and drainage water, while municipal and industrial discharges can create localized point-source concentrations.[7,9] Once released, TFA resists degradation under ordinary environmental conditions. Its small size and weak hydrophobicity also limit removal by conventional biological treatment, activated carbon, and other technologies.[5,7]

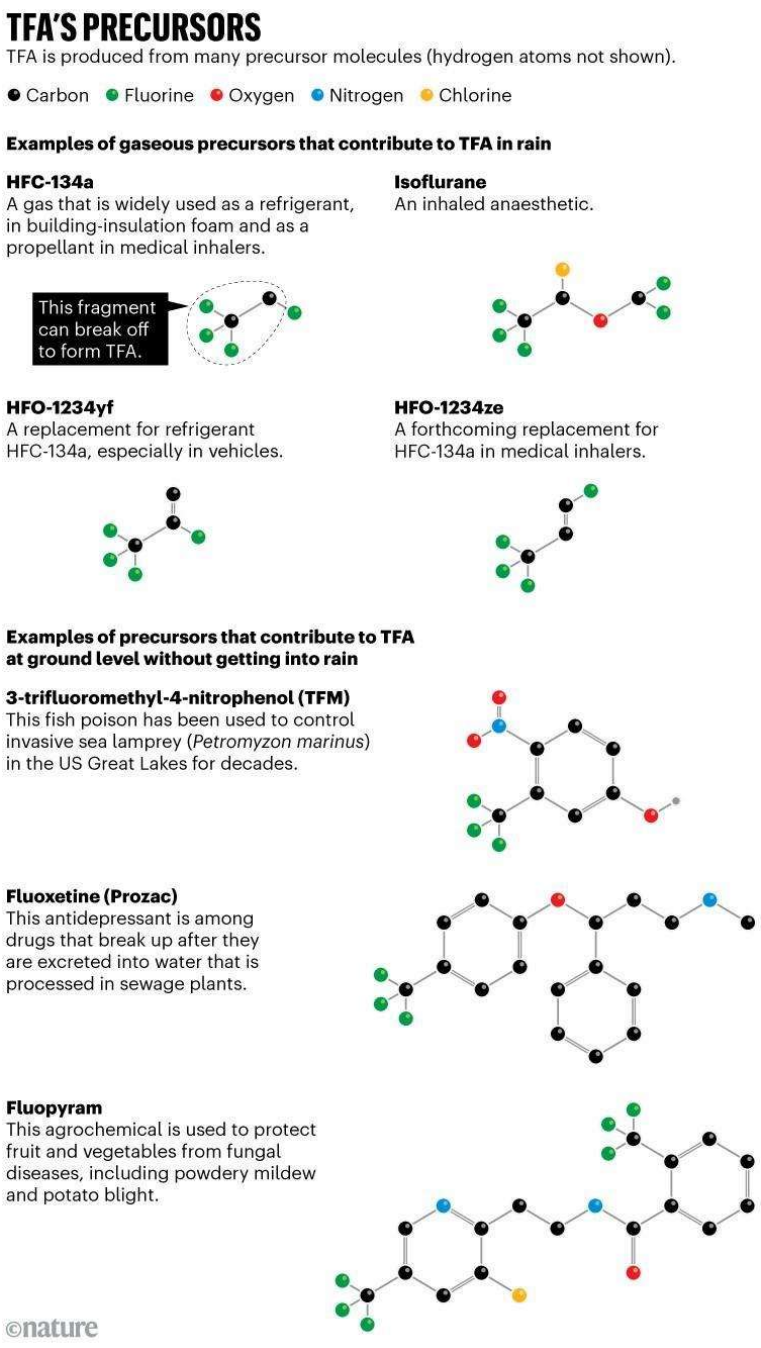

Figure 2 TFA precursors
Figure from Lim, X. There's a new acid in our rain — should we be worried? Nature.2025 643 (8073), 894-897 https://www.nature.com/articles/d41586-025-02259-6

1.4 Occurrence in Surface Water and Related Matrices

Monitoring studies increasingly identify TFA as a major contributor to measured PFAS mass in water. Published data reviewed by Arp et al.[10] show increasing concentrations across multiple media and report that TFA can exceed the combined mass of many routinely monitored PFAS.[10]

A 2024 European survey detected TFA in all 23 surface-water and six groundwater samples analyzed, with reported concentrations from 370 to 3,300 ng/L.[11] These results provided some additional evidence that TFA is widespread in European waters; however, the survey was conducted by a nongovernmental organization rather than reported as a peer-reviewed monitoring study. Therefore, when interpreting the reported concentration, the analytical methods and quality assurance information, such as sample collection procedures, calibration procedures, and blank contamination controls should be considered.

CHAPTER 2: HEALTH, ENVIRONMENTAL, AND REGULATORY SIGNIFICANCE

2.1 Health and Ecological Concerns

Research on the human health effects of long-chain PFAS is significantly more comprehensive than that for TFA.[3,10] Therefore, it would be inappropriate to automatically apply all long-chain PFAS hazard conclusions to ultrashort-chain compounds. Moreover, it is equally inappropriate to conclude that high mobility and lower aquatic bioaccumulation imply negligible risk. Risk depends on both hazard and exposure, and persistent emissions can lead to lifelong, widespread exposure even when individual concentrations are lower than those associated with acute toxicity.[3,10]

Recent evaluations of TFA have identified potential liver effects and reproductive or developmental toxicity in mammalian studies, highlighting significant uncertainties regarding dose-response interpretations, sensitive endpoints, vulnerable populations, and chronic low-dose exposure.[10] More recent experimental evidence has also identified eye malformations following developmental TFA exposure in rabbits. [12] This finding is notable because adverse effects on eye development had not previously been recognized as a prominent toxicological endpoint for TFA.[12] The findings contribute to increased regulatory concern in Europe. In June 2026, the ECHA Committee for Risk Assessment concluded that TFA should be classified as reproductive toxicant Category 1B for development effects (Repr. 1B; H360D).[13] There is relatively limited ecotoxicity data, especially concerning terrestrial plants and multigenerational exposure. Additionally, TFA uptake by plants and its presence in food and beverages expand the potential exposure pathways beyond just drinking water.[10]

2.2 Health-Based Values and Regulatory Context

Health-based and precautionary limits for TFA differ among jurisdictions because they rely on different toxicological datasets, uncertainty factors, and exposure assumptions. The German Federal Environment Agency established a health-based drinking-water guideline of 60 µg/L and recommended keeping concentrations below 10 µg/L where reasonably achievable.[14] The Netherlands derived an indicative value of 2.2 µg/L, while Denmark established a value of 9 µg/L.[15] These differences demonstrate both the evolving nature of the evidence and the importance of comparable analytical data. Regulatory treatment of TFA is further complicated by differences in how agencies and jurisdictions define PFAS. For example, the U.S. EPA working definition used for its 2021 National PFAS Testing Strategy required a structure containing at least two adjacent fluorinated carbon atoms and therefore excluded TFA, since it contains only a single carbon atom.[16] This contrasts with broader definitions and regulatory approaches and contributes to whether TFA is included in PFAS monitoring, assessment, and regulatory programs.

TFA is not included in many routine PFAS regulatory methods and compound lists.[5,15] Consequently, conventional PFAS monitoring programs may not capture TFA even when it is present at concentrations greater than those routinely monitored PFAS.[10,15] Therefore, method availability influences which contaminants monitoring programs can detect and which contaminants receive regulatory attention.

Efforts to standardize analytical measurement of TFA in water have advanced through the development of formal and proposed LC-MS/MS procedures. The draft DIN 38407-53 method describes direct injection LC-MS/MS analysis of TFA in water.[15] The California CAC-TFA-1.1 method establishes additional quality-control and methods-performance requirements for TFA measurement in water.[17] An interlaboratory trial involving 12 laboratories reported relative

repeatability standard deviations of 3-7% and reproducibility standard deviations of 6-20% across drinking water, groundwater, surface water, rainwater, and fortified samples. [15]

2.3 Need for Targeted Method Development and Evaluation

Environmental monitoring requires selective, accurate, and precise measurements. For TFA, apparent differences among samples may be influenced by matrix suppression, laboratory contamination, calibration bias, inadequate chromatographic retention, or variable instrumental response.[5,18]

Developing and preliminarily evaluating a targeted method can establish whether an analytical approach provides reproducible chromatographic retention, suitable calibration behavior, and measurable response over the intended concentration range. Furthermore, a formal validation requires independent evaluation of accuracy, precision, recovery, matrix effects, and detection or quantitation limits.[15,17]

Current methods that are optimized primarily for long-chain PFAS may provide inadequate retention of TFA.[5,18] Therefore, the present study focuses on development and preliminary evaluation of a HILIC-LC-MS/MS approach as a foundation for subsequent formal validation and environmental application.

CHAPTER 3: ANALYTICAL BACKGROUND AND METHOD DEVELOPMENT

3.1 Limitations of Conventional Reversed-Phase Methods

Most routine PFAS LC-MS/MS methods use reversed-phase C18 chromatography and weak-anion-exchange solid-phase extraction. These approaches perform well for many C4 and longer analytes because retention increases with hydrophobic chain length.[5] TFA has insufficient hydrophobic interaction with conventional stationary phases and often elutes near the void volume with salts and other highly polar constituents.[5] This can result in poor peak shape, unstable retention, low sensitivity, and severe electrospray ionization suppression.[5]

Offline solid-phase extraction can improve sensitivity for many PFAS but may provide low or variable recovery for ultrashort-chain acids.[5] Additional preparation steps also create opportunities for contamination and analyte loss. Direct injection reduces sample handling, consumable use, and contamination risk but yields higher detection limits because it omits a sample concentration step.[5, 18] Because the sample matrix is introduced directly into the LC-MS/MS system, the chromatographic method must provide sufficient retention and separation of TFA from salts and other highly polar co-eluting constituents, while the mass-spectrometric response must remain sufficiently robust to potential matrix-induced ion suppression.[5, 18]

3.2 Alternative Chromatographic Strategies

Alternative chromatographic strategies for ultrashort chain PFAS include supercritical fluid chromatography, HILIC, hybrid HILIC/ion-exchange phases, and trap-column configurations.[5, 18–20] These approaches provide greater retention or selectivity for highly polar and ionic analytes.

Strong ion-exchange methods can retain highly ionic analytes but may require long runtimes or produce broad peaks.[5] Supercritical fluid chromatography can provide useful selectivity for highly polar fluorinated compounds, but it requires specialized instrumentation.[5,19] Mixed-mode and hybrid HILIC/ion-exchange phases combine polar partitioning and ionic interactions and have shown particular promise for ultrashort-chain PFAS.[18]

Liang et al.[18] reported a seven-minute direct-injection method for ultrashort-chain PFAS using a hybrid HILIC/ion-exchange column. Across tap water, bottled spring water, and wastewater, reported recoveries were 86.6-107% with relative standard deviations of 1.62-10.7%. TFA required a higher calibration starting concentration, illustrating compound-specific sensitivity within the same ultrashort-chain method.[18]

3.3 Tandem Mass Spectrometry Considerations

TFA is analyzed effectively using negative electrospray ionization because it is a strong acid and occurs as an anion under typical aqueous analytical conditions.[5, 15] Triple-quadrupole mass spectrometry provides selectivity through multiple-reaction monitoring (MRM), in which a precursor ion is selected, fragmented, and monitored through one or more product-ion transitions.[15, 18] Low molecular mass limits the number of structurally distinctive fragments, particularly for TFA, making chromatographic separation, retention-time stability, and blank control crucial to identification.

Published TFA methods commonly monitor the transition near m/z 113 to 69 for native TFA and an offset transition for $^{13}C_2$-labeled TFA.[15, 17, 18] Final transitions, collision energies, fragmentor voltages, dwell times, and source conditions must be verified on the specific instrument using standards and sufficient data points across each chromatographic peak.[18,21,22]

In this study, an Agilent 6470 triple-quadrupole LC-MS/MS system was used for method development. MassHunter acquisition and quantitative-analysis software were used for MRM method development, optimization, calibration, peak integration, and chromatographic review.[21, 22]

CHAPTER 4: MATERIALS AND METHODS

4.1 Study Design and Method Evaluation Objectives

This study aimed to develop and preliminarily evaluate an LC-MS/MS method to quantitatively determine TFA in laboratory-prepared aqueous samples. Method development focused on establishing reproducible chromatographic retention, optimizing the selected mass-spectrometric transition, and evaluating calibration behavior. Other factors such as characterizing laboratory background, assessing potential carryover, and estimating analytical sensitivity are also examined.

Selectivity was evaluated by comparing TFA standards with laboratory blanks using the selected MRM transition (m/z 113 → 69) and chromatographic retention time. Calibration performance was evaluated using the relationship between nominal concentration and instrumental response, coefficient of determination (R^2), concentrations calculated by MassHunter from the fitted calibration curve, and relative difference between calculated and nominal standards.[23]

The study was intended as a preliminary evaluation rather than a complete formal method validation. Independent replicate samples designed specifically to establish method accuracy, precision, recovery, and matrix-specific performance were not analyzed. These parameters therefore remain subjects for subsequent validation work.

4.2 Chemicals, Standards, and Reagents

The primary stock, intermediate, and calibration solutions were prepared using Neat TFA. LC-MS grade water was used for aqueous solution preparation, and LC-MS grade acetonitrile was used as the organic mobile-phase component.

Table 1 Chemicals and Reagents

Reagent	Supplier	Catalog No.	Lot No.	Purity	Form	CAS No.
TFA	Fisher Chemical	A116-05AMP	234728	≥ 99.5%	Liquid (acid)	76-05-01
LCMS Water	Fisher Chemical	W6500	254706	-	Liquid	7732-18-5
Acetonitrile	Fisher Chemical	A955-4	215182	≥ 99.9%	Liquid	75-05-8

4.3 Preparation of Stock and Working Standards

The primary stock solution was prepared from neat TFA reagent with a purity of ≥ 99.5% and was first converted to a mass-based concentration using the reagent's purity and density. The intermediate and working solutions were prepared by using serial volumetric dilution with LC-MS grade water using the following dilution relationship

$$C_1V_1 = C_2V_2$$

where (C_1) represents the concentration of the source solution, (V_1) is the required source volume, (C_2) is the desired concentration, and (V_2) is the final solution volume. The primary stock to prepare intermediate and working standards was prepared using serial dilutions.

Intermediate and working solutions were stored in Thermo Fisher Scientific virgin polypropylene 1-10 mL tubes at approximately 10 °C for no longer than 30 days. Low-level working standards were prepared bi-weekly to reduce concerns about adsorption, degradation, and contamination.

The calibration standards used for the current calibration curve were prepared at concentrations of 75, 100, 125, 150, and 300 ng/L. Additional working solutions were prepared during method development as needed to optimize and assess instrumental response. Appendix A provides the complete preparation scheme and volumes used for TFA stock and working solutions.

4.4 Scope of Preliminary Method Evaluation

The preliminary method evaluation used laboratory-prepared aqueous TFA standards in LC-MS-grade water rather than authentic environmental surface-water samples. Using a controlled aqueous system allowed examination of chromatographic behavior, instrumental response, calibration performance, and laboratory background without additional variability from dissolved organic matter, suspended solids, variable ionic strength, and other environmental matrix constituents. [18]

The results therefore characterize analytical performance under controlled laboratory conditions and should not be interpreted as a complete method validation or as evidence of quantitative performance in authentic surface-water matrices. Formal validation using independent replicate samples and representative environmental matrices is required before routine environmental application.

4.5 LC-MS/MS Instrumentation

Analyses were performed using an Agilent 6470 triple-quadrupole LC-MS/MS system equipped with an electrospray ionization source operated in negative-ion mode. MassHunter software, including Data Acquisition, Qualitative Analysis 10.0, and QQQ quantitative analysis, was used for acquisition, optimization, integration, calibration, and quantitative analysis. [21, 22] The LC system was tuned weekly using autotune methods. Before analysis, the LC system and HILIC column were conditioned by flushing the column for 35 minutes with 80% acetonitrile and 20% water, followed by 15 minutes of pump purging and 10 minutes of pump-head conditioning before each batch. A 30-minute post-run flush was also performed with 80% acetonitrile and 20% water.

4.6 Chromatographic Conditions

Chromatographic separation was performed using an Agilent AdvanceBio HILIC column (2.1 x 150 mm, 2.7 µm particle size). Mobile phase A consisted of 2 mM ammonium acetate in LC-MS grade water, and mobile phase B was acetonitrile. The column temperature was maintained at 40 °C, with a flow rate of 0.15 mL/min and an injection volume of 1 µL. The total analytical run time was 9.5 minutes.

A gradient elution setup was used to promote retention and subsequent elution of the highly polar TFA analyte on the HILIC stationary phase. HILIC and hybrid HILIC/ion-exchange approaches have previously demonstrated improved retention of ultrashort-chain PFAS relative to conventional reversed-phase chromatography.[5,18] The method began at 5% mobile phase A and 95% mobile phase B. This condition was held for 2.0 minutes. The mobile-phase A proportion was then increased to 20% at 4.5 minutes and 30% at 5.5 minutes, and maintained through 6.0 minutes. At 6.6 minutes, the mobile-phase composition was returned to the initial condition and maintained through 9.5 minutes. Under these chromatography conditions, TFA eluted reproducibly at approximately the 2.20 minute mark.

Table 2 LC conditions

Parameter	Current/Final Value
Column	Agilent AdvanceBio HILIC, 2.1 x 150 mm 2.7-µm
Mobile phase A	2mM ammonium acetate in LC-MS grade Water
Mobile phase B	Acetonitrile
Flow rate	0.15 mL/min
TFA retention time	2.20 minutes
Injection volume	1 µL
Column temperature	40 °C
Run time	9.5 minutes

Table 3 Elution gradient

Time (min)	A (%)	B(%)
0.00	5	95
2.00	5	95
4.50	20	80
5.50	30	70
6.00	30	70
6.60	5	95
9.50	5	95

4.7 Mass Spectrometric Conditions

The 5 ppb TFA standard was used to optimize TFA mass-spectrometric conditions, and the analysis was done using negative electrospray ionization and multiple-reaction monitoring. The transition m/z 113 → 69 was selected as the final quantitative transition for TFA, consistent with published TFA LC-MS/MS approaches.[15, 17, 18] Optimized acquisition parameters included a fragmentor voltage of 83 V, collision energy of 12 V, and dwell time of 100 ms.

During method development, the dwell time increased from an initial setting of 10 ms to 100 ms. Increasing the dwell time increased the amount of acquisition time devoted to the selected TFA transition and improved the measurable response for the low-abundance analyte signal. Because the final method monitored a limited number of transitions, a longer dwell time could be used while maintaining sufficient measurements across the chromatographic peak.

TFA eluted at approximately 2.20 min under the developed chromatographic conditions.

Table 4 MRM transitions and optimized parameters.

Analyte	Precursor	Product	Role	Fragmentor	CE	Dwell	RT
TFA	113	69	Quantifier	83	12	100	2.2

4.8 Calibration and Quantification

The TFA calibration standards in LC-MS-grade water were prepared at nominal concentrations of 75, 100, 125, 150, and 300 ng/L. Instrument response was quantified using the integrated chromatographic peak area for the TFA m/z 113 → 69 transition.

The linear calibration models were evaluated using 1/x and $1/x^2$ weighting. Weighted regression was examined because analytical variance can increase with concentration and because excessive influence from higher-concentration standards may reduce calibration performance near the lower end of the analytical range. Selection of the calibration model considered the relationship between concentration and response, calculated concentrations of calibration standards, and performance across the working range rather than relying solely on R^2.[23]

For the calibration batch presented in this study, the 1/x-weighted model produced a calibration equation of ($y = 20810.89x - 290.53$) and ($R^2 = 0.9972$). The $1/x^2$-weighted model produced ($y = 21160.50x - 332.82$) and ($R^2 = 0.9945$).

4.9 Quality Assurance and Quality Control

During method development, quality assurance and quality control focused on monitoring laboratory background, calibration performance, chromatographic consistency and potential carryover. Laboratory blanks were analyzed throughout the analytical sequence and were evaluated for measurable responses at or near the TFA retention time. Blank responses were compared with those of the calibration standards to assess potential interference with TFA detection at the lower end of the calibration range.

Calibration standards were reviewed for consistency between nominal concentration and the concentration values from MassHunter (Agilent Quantification Software). For each standard,

the chromatographic retention time and peak integration were examined. TFA retention time remained approximately 2.20 minutes across the calibration sequence.

The California CAC-TFA-1.1 method was used as a reference for general quality-control considerations for TFA analysis.[17] However, the complete formal validation procedures described in that method were not performed in the present study.

4.10 Sensitivity Calculation

Laboratory blank variability and the slope of the selected TFA calibration curve were used to evaluate the sensitivity. The limit of blank (LoB), limit of detection (LoD), and limit of quantitation (LoQ) represent related but distinct levels of analytical performance. The LoB describes the highest apparent analyte response expected from repeated analysis of samples containing no analyte. The LoD represents the lowest analyte concentration that can be distinguished from analytical background with reasonable confidence, while the LoQ represents the lowest concentration at which the analyte can be quantitatively measured with acceptable analytical performance.[24]

Armbruster and Pry describe these concepts using the statistical distributions of blank and low-concentration samples and emphasize that detecting an analyte does not necessarily demonstrate that it can be reliably quantified.[24] In the present study, replicate laboratory blanks were available to characterize background response; however, replicate low-concentration TFA samples prepared specifically near the detection and quantitation limits were not analyzed. Therefore, a full experimental LoB/LoD/LoQ assessment based on both blank and low-concentration sample distributions was not performed.

Instead, the approach described in the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) Q2(R2) guideline, Validation

of Analytical Procedures, was used to estimate preliminary detection and quantitation limits. [23] The estimated detection limit was calculated as:

$\text{LoD} = \frac{3.3\,\sigma}{S}$; $\text{LoQ} = \frac{10\,\sigma}{S}$ [23]

where (σ) represents the standard deviation of the analytical response from laboratory blanks and (S) represents the slope of the calibration curve.[23] ICH Q2(R2) allows detection and quantitation limits to be estimated from response variability and calibration sensitivity, while also recognizing the importance of confirming analytical performance near the estimated lower concentration range.[23]

Because replicate TFA samples prepared specifically near the calculated detection and quantitation limits were not analyzed, the resulting LoD and LoQ values were interpreted as preliminary estimates of analytical sensitivity rather than formally validated method limits.

CHAPTER 5: RESULTS AND DISCUSSION

5.1 Method Optimization

Development of the TFA method focused on achieving reproducible chromatographic retention and adequate LC-MS/MS response for an analyte that is poorly retained by conventional reversed-phase chromatography.[5,18] An Agilent AdvanceBio HILIC column was selected to increase retention of the highly polar TFA anion under high-organic chromatographic conditions. Method development included adjustment of mobile-phase composition, flow conditions, injection parameters, run time, and column-conditioning procedures.

Mass-spectrometric optimization identified m/z 113 → 69 as the primary TFA transition. A fragmentor voltage of 83 V and collision energy of 12 V were selected. The acquisition dwell time was initially 10 ms and was subsequently increased to 100 ms. The longer dwell time increased the acquisition time allocated to the TFA transition and improved the analyte response. The resulting chromatographic peaks exhibited some fine-scale signal variation; however, a distinct and reproducible peak remained evident and could be consistently integrated. Because preliminary quantitative assessment depended on reproducible peak integration, retention time, and calibration response rather than visual smoothness alone, the 100 ms dwell condition was retained for subsequent analysis.

The developed method produced a TFA retention time of approximately 2.20 min within a total run time of 9.5 min. During final method development, the HILIC column experienced elevated pressure (> 550 bar), requiring additional column conditioning and evaluation of flow and mobile-phase conditions.

5.2 Selectivity, Blank Contamination, and Carryover

Representative chromatograms for the laboratory blank and TFA standards are shown in Figure 3. Distinct chromatographic responses were observed for the TFA standards near the established retention time of approximately 2.20 min. Retention time was highly consistent across the five calibration concentrations, ranging from 2.196 to 2.201 min for standards between 75 and 300 ng/L.

Blank responses were more than two orders of magnitude lower than responses from the calibration standards. Observed blank peak areas were generally between 1 and 11, whereas the 75 ng/L calibration standard produced an area of 1,206 and the 300 ng/L standard produced an area of 5,907. Calculated blank concentrations were correspondingly near zero relative to the calibration range.

A blank analyzed immediately after the 300 ng/L calibration standard produced a peak area of 2 compared with an area of 5,907 for the preceding standard. The response in this post-calibration blank was therefore approximately 0.034% of the 300 ng/L standard response. No substantial increase in blank response was therefore observed immediately following the highest calibration standard in this sequence. These observations indicate low analytical background and no evident substantial carryover within the evaluated calibration batch. Control of laboratory background is particularly important for TFA because standardized methods explicitly incorporate method blanks to assess potential background contamination.[17]

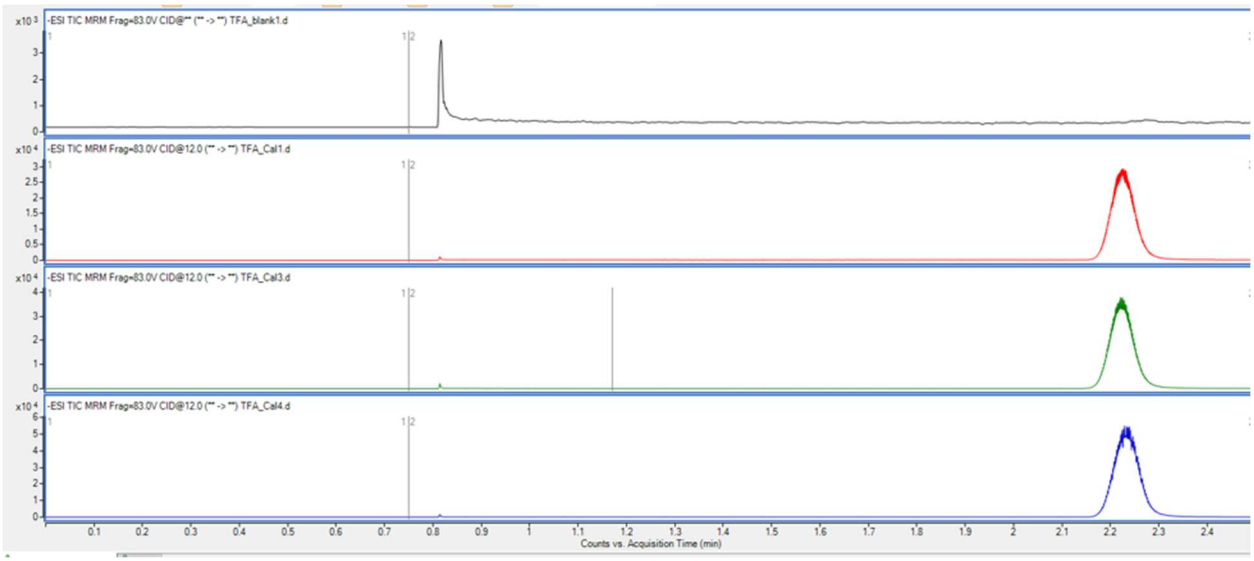

Figure 3 Representative TFA MRM chromatograms for a laboratory blank and 75, 125, and 150 ng/L standards, shown from top to bottom.

5.3 Calibration Performance and Linearity

This study evaluated calibration performance using five TFA standards at concentrations of 75, 100, 125, 150, and 300 ng/L. Instrumental response increased with increased concentration, with peak areas of 1,206, 1,886, 2,278, 2,879, and 5,907.

The 1/x-weighted linear regression produced the calibration equation (y = 20810.89x - 290.53) with (R^2 = 0.9972). The $1/x^2$-weighted model produced (y = 21160.50x - 332.82) with (R^2 = 0.9945). Both models demonstrated a strong relationship between TFA concentration and instrumental response; however, the 1/x model produced the higher coefficient of determination. Although R^2 was used as one indicator of calibration behavior, the calculated concentrations of individual standards were also used to assess model performance across the evaluated range.[23]

Using the 1/x calibration, back-calculated concentrations for the 75, 100, 125, 150, and 300 ng/L standards were 63.9, 99.9, 120.7, 152.5, and 313.0 ng/L, respectively. The calculated concentrations corresponded to 85.2%, 99.9%, 96.6%, 101.7%, and 104.3% of their respective

nominal concentrations. Relative errors were −14.8%, −0.1%, −3.4%, +1.7%, and +4.3%, respectively.

The greatest deviation occurred at the lowest calibration level (75 ng/L), indicating greater analytical uncertainty near the lower boundary of the current calibration range. In contrast, standards between 100 and 300 ng/L were within approximately 5% of their nominal concentrations. These results support use of the 1/x-weighted linear model for the evaluated calibration range.

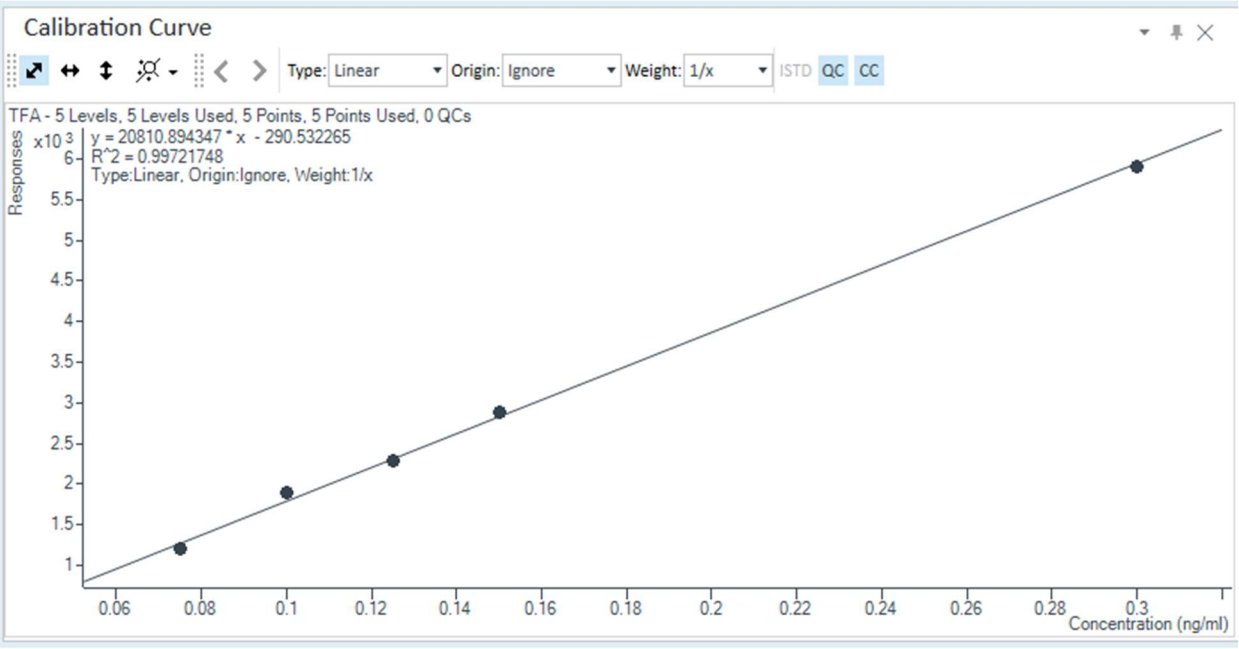

Figure 4 TFA Calibration Curve (1/x)

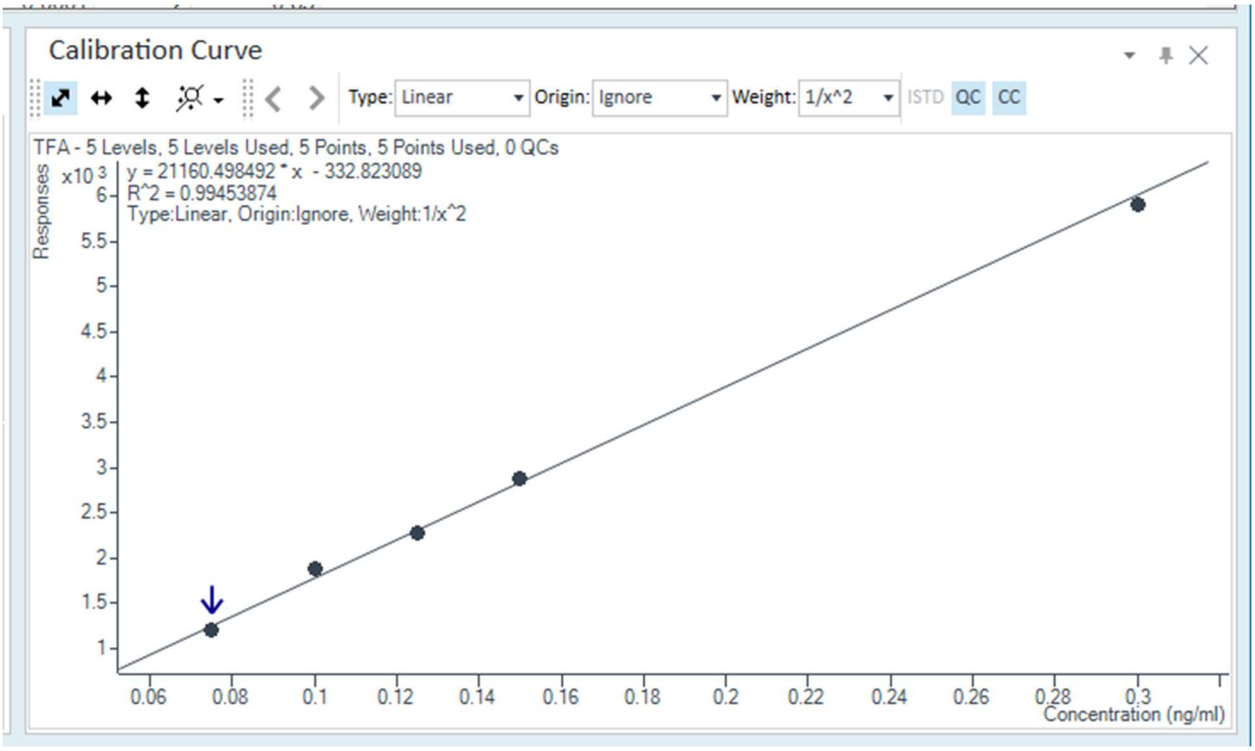

Figure 5 TFA Calibration Curve ($1/x^2$)

Batch Table

Sample: Blank Sample Type: <All> Compound: TFA ISTD:

Sample				TFA Meth...	TFA Results					
Name	Data File	Type	Level	Exp. Conc.	RT	Resp.	MI	Calc. Conc.	Final Conc.	Area	S/N
Blank	TFA_blank9.d	Blank			2.210	1		0.0000	0.0000	1	0....
Blank	TFA_blank1.d	Blank			2.205	2		0.0001	0.0001	2	0....
Blank	TFA_blank2.d	Blank			2.192	4		0.0002	0.0002	4	0....
Blank	TFA_blank3.d	Blank			2.191	3		0.0002	0.0002	3	0....
Blank	TFA_blank4.d	Blank			2.197	1		0.0001	0.0001	1	0....
Blank	TFA_blank5.d	Blank			2.203	4		0.0002	0.0002	4	1....
Blank	TFA_blank6.d	Blank			2.204	2		0.0001	0.0001	2	0....
Blank	TFA_blank7.d	Blank			2.204	2		0.0001	0.0001	2	0....
Blank	TFA_blank8.d	Blank			2.207	3		0.0001	0.0001	3	0....
Calibration 75 ppt	TFA_Cal1.d	Cal	1	0.0750	2.201	1206		0.0639	0.0639	1206	3...
Calibration 100 ppt	TFA_Cal2.d	Cal	2	0.1000	2.196	1886		0.0999	0.0999	1886	3...
Calibration 125 ppt	TFA_Cal3.d	Cal	3	0.1250	2.200	2278		0.1207	0.1207	2278	2...
Calibration 150 ppt	TFA_Cal4.d	Cal	4	0.1500	2.200	2879		0.1525	0.1525	2879	2...
Calibration 300 ppt	TFA_Cal7.d	Cal	5	0.3000	2.201	5907		0.3130	0.3130	5907	4...
Blank	TFA_blank10.d	Blank			2.207	2		0.0001	0.0001	2	0....
Blank	TFA_blank11.d	Blank			2.211	7		0.0004	0.0004	7	0....
Blank	TFA_blank12.d	Blank			2.251	1		0.0001	0.0001	1	0....
Blank	TFA_blank13.d	Blank			2.196	2		0.0001	0.0001	2	0....
Blank	TFA_blank14.d	Blank			2.233	11		0.0006	0.0006	11	1....
Blank	TFA_blank15.d	Blank			2.211	1		0.0000	0.0000	1	0....
Blank	TFA_blank16.d	Blank			2.220	6		0.0003	0.0003	6	1....
Blank	TFA_blank17.d	Blank			2.204	2		0.0001	0.0001	2	0....

Figure 6 Calibration Performance

Table 5 Calibration performance summary.

Analyte	Range (ng/L)	Model	Weighting	Equation	R^2
TFA	75 – 300	Linear	1/x	20810.89x -290.53	0.9972
TFA	75 – 300	Linear	$1/x^2$	21160.50x -332.82	0.9945

5.4 Limitation of Preliminary Laboratory Water Sample Evaluation and Matrix Effects

The present study used laboratory-prepared standards in LC-MS-grade water and therefore did not directly evaluate matrix effects associated with authentic environmental surface water. Surface-water samples may contain dissolved organic matter, inorganic ions, suspended solids, and co-occurring compounds that can affect quantitative LC-MS/MS performance.[17,18]

Using a controlled aqueous system allowed evaluation of chromatographic behavior, calibration response, blank contamination, and preliminary analytical sensitivity without environmental matrix variability. However, the resulting data cannot establish quantitative accuracy, precision, or recovery in authentic environmental samples.

The performance characteristics reported in this study should therefore be interpreted as preliminary analytical observations under controlled laboratory conditions. Future matrix-specific validation using representative surface-water samples will be required to determine whether ion suppression, ion enhancement, sample composition, or other matrix-related effects influence quantitative performance.

5.5 Estimated Detection and Quantitation Limits

Seventeen laboratory blank injections were evaluated. Blank peak areas ranged from 1 to 11, with a mean response of 3.18 and a standard deviation of 2.65. The 1/x weighted TFA calibration produced a slope of 20,810.89 response units per ng/mL. Using these values, the estimated LoD was approximately 0.00042 ng/mL (0.42 ng/L), and the estimated LoQ was approximately 0.00127 ng/mL (1.27 ng/L).

These estimates are substantially below the lowest experimentally evaluated calibration standard of 75 ng/L. Because replicate low-concentration TFA samples near the calculated limits were not analyzed, interpret these values as preliminary estimates of analytical sensitivity rather than validated method detection and quantitation limits.[23,24]

5.6 Implications for Future Surface-Water Application

The developed method was intended as a foundation for future determination of TFA in environmental surface water. The present work establishes chromatographic retention, MS/MS detection conditions, calibration behavior, laboratory blank characteristics, carryover behavior, and preliminary analytical sensitivity under controlled laboratory conditions.

Application to environmental surface water will introduce additional matrix variability not evaluated in the present study. Published TFA methods have therefore evaluated performance using authentic drinking-water, groundwater, surface-water, and wastewater matrices and have incorporated approaches such as matrix fortification and isotopically labeled internal standards.[15,17,18]

5.7 Comparison with Published Methods

Published methods for determination of TFA and other ultrashort-chain PFAS have employed HILIC or hybrid HILIC/ion-exchange chromatography, direct injection, trap-column enrichment, and other strategies to overcome the poor reversed-phase retention of highly polar analytes.[5,18,20]

Liang et al.[18] developed a direct-injection hybrid HILIC/ion-exchange LC-MS/MS method for C1–C4 PFAS with a TFA calibration range of 20–800 ng/L. Across tap water, bottled spring

water, and wastewater, recoveries ranged from 86.6 to 107%, with relative standard deviations of 1.62–10.7%.

The California CAC-TFA method similarly uses direct-injection LC-MS/MS and monitors the TFA transition m/z 113 → 69.[17] The California multi-laboratory study required a minimum five-point calibration and reported laboratory-specific precision values ranging from 1.4 to 14.5% RSD during initial demonstration of capability.

The method developed in the present study used the same principal TFA transition, m/z 113 → 69, with HILIC separation and a 75–300 ng/L calibration range. TFA eluted reproducibly at approximately 2.20 min, and the 1/x-weighted calibration produced an R^2 of 0.9972. Because independent accuracy, precision, recovery, and matrix-specific validation were not performed, direct comparison of those formal performance characteristics with fully validated published methods is not appropriate.

The evaluated 75–300 ng/L calibration range overlaps concentrations reported in several environmental water studies,[25–28] indicating that the developed method covers a portion of the environmentally relevant TFA concentration range, although higher-concentration samples would require the calibration-range to be extended prior to analysis.[25–28]

CHAPTER 6: CONCLUSION

This thesis addressed the analytical challenges associated with measurement of trifluoroacetic acid (TFA), a highly mobile and persistent ultrashort-chain PFAS that can be poorly retained by conventional reversed-phase analytical methods.[5,10,18] A targeted LC-MS/MS method was developed using HILIC chromatography and negative electrospray-ionization tandem mass spectrometry.

The developed method used the TFA transition m/z 113 → 69 with an optimized fragmentor voltage of 83 V, collision energy of 12 V, and dwell time of 100 ms. TFA exhibited reproducible chromatographic retention at approximately 2.20 min under the selected HILIC conditions. Calibration over the range of 75–300 ng/L produced a concentration-dependent instrument response. The 1/x-weighted linear regression produced an R^2 of 0.9972, and the concentrations calculated by MassHunter for standards between 100 and 300 ng/L were within approximately 5% of their nominal concentrations. The lowest calibration level (75 ng/L) showed a greater deviation of approximately 15%, indicating increased quantitative uncertainty near the lower boundary of the evaluated calibration range.

Laboratory blank responses were more than two orders of magnitude lower than the response obtained for the lowest calibration standard. A blank analyzed immediately after the 300 ng/L calibration standard produced a response equivalent to approximately 0.034% of the preceding standard, indicating minimal observable carryover under the evaluated analytical conditions.

Preliminary estimates of analytical sensitivity were calculated using laboratory blank variability and the slope of the 1/x-weighted calibration curve according to the calibration-slope approach described in ICH Q2(R2).[23] The estimated LoD and LoQ were approximately 0.42 and

1.27 ng/L, respectively. These estimates are substantially below the lowest experimentally evaluated calibration standard of 75 ng/L and were not confirmed using replicate low-concentration TFA samples. Therefore, they should be interpreted as preliminary estimates of analytical sensitivity rather than validated method detection and quantitation limits.[23,24]

Published environmental measurements show that the 75-300 ng/L calibration range evaluated in this study overlaps with TFA concentrations reported in various water matrices. For example, a resampling study of streams in Northern California reported a median TFA concentration of 180 ng/L, with concentration ranges from 21.3 to 2,790 ng/L.[25] A more recent study has reported TFA concentrations ranging from 41- 315 ng/L in surface water and 51- 123 ng/L in drinking water. These findings further suggest that the current calibration range is environmentally relevant.[26] In groundwater, concentrations of at least 100 ng/L have been reported in samples recharged after 1980, with concentrations increasing in more recently recharged water.[27]

However, a 2024 European survey reported TFA concentrations in the range of 370-3,300 ng/L in surface and groundwater,[11] while other studies have reported concentrations exceeding 100 µg/L in surface water affected by industrial discharges.[7,28] The current calibration range captures relatively low to moderate concentrations of TFA in the environment; therefore, it would require extension for more elevated TFA concentrations.

Overall, the results demonstrate the feasibility of the developed HILIC-LC-MS/MS approach for TFA measurement in controlled aqueous samples. The study established reproducible chromatographic retention, optimized MS/MS acquisition conditions, a concentration-dependent calibration response, low laboratory blank response, and preliminary estimates of analytical sensitivity. However, independent evaluation of accuracy, precision, recovery, matrix effects, and

low-level quantitative performance was outside the scope of the present study. Formal validation using replicate samples and representative environmental matrices will therefore be required before the method can be considered validated for routine surface-water monitoring.[15,17,18]

CHAPTER 7: FUTURE WORK

Future work should first focus on formally validating the developed TFA method. Independent replicate samples should be analyzed across the intended quantitative range to establish accuracy, precision, recovery, and reproducibility. Published and standardized TFA methods use replicate fortified samples, calibration-verification standards, blanks, and other quality-control samples to establish analytical performance.[15,17] Particular attention should be given to concentrations near the lower end of the analytical range so that detection and quantitation limits can be confirmed experimentally rather than estimated solely from calibration slope and blank variability.[23,24]

Following laboratory validation, the method should be evaluated using authentic environmental surface-water matrices. Surface waters representing different ionic strengths, dissolved organic carbon concentrations, suspended-solid contents, and potential anthropogenic influences would provide a broader assessment of method robustness. Published ultrashort-chain PFAS methods show that analytical performance can vary across water matrices, emphasizing the importance of matrix-specific evaluation.[17,18] Stormwater, post-fire runoff, reservoir water, river water, and wastewater-impacted surface water would therefore provide useful matrices for subsequent testing.

Matrix effects should be evaluated using approaches such as matrix fortification, matrix-matched calibration, standard addition, or comparison with isotopically labeled TFA internal standards. Published TFA methods commonly use isotopically labeled internal standards to compensate for variability associated with sample preparation, electrospray-ionization response, and instrumental analysis.[15,17,18] A compound-specific isotopically labeled TFA internal standard

would therefore be valuable for evaluating and correcting potential signal suppression or enhancement in environmental samples.

Additional studies should evaluate sample holding time, freeze-thaw stability, filtration effects, and potential adsorption or contamination associated with different container materials. Laboratory sources of TFA background should also continue to be evaluated because ultrashort-chain PFAS analysis can be sensitive to contamination from analytical water, solvents, tubing, seals, and other laboratory materials.[5] These experiments would help determine whether storage and handling conditions influence measured TFA concentrations before routine environmental application.

Further chromatographic optimization should evaluate long-term HILIC column pressure stability, column-conditioning requirements, and the effects of mobile-phase composition and flow rate on TFA retention and system backpressure. The elevated backpressure observed during later stages of the present method-development work demonstrates the importance of establishing stable operating conditions before running larger analytical batches.

Interlaboratory evaluation would provide an additional measure of method reproducibility across instruments, analysts, columns, and laboratories. Previous TFA interlaboratory work has demonstrated that comparable LC-MS/MS measurements can be achieved across multiple laboratories while also identifying variability associated with laboratory-specific analytical conditions.[15] Such testing would be an important subsequent step if the method developed in this study were intended for broader environmental monitoring applications.

Finally, after formal TFA validation is complete, the analytical scope could be expanded to additional ultrashort-chain PFAS. HILIC, hybrid HILIC/ion-exchange, and other polar chromatographic strategies have already demonstrated the capability to retain multiple C1–C4

PFAS in water.[5,18,20] Expansion to additional analytes should therefore be evaluated while maintaining chromatographic retention, sensitivity, and quantitative performance for TFA.

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APPENDIX A: TFA Solution Preparation Table

Solution/Level	Source Concentration	Target Concentration	Volume of source	Dilution Volume (LCMS water)	Final Volume
Intermediate Stock Solution 1	1.482×10^6 ppm Neat TFA	1000 ppm	67.5 μL	99.9325 mL	100 mL
Intermediate Stock Solution 2	1000 ppm	1000 ppb	100 μL	99.9 mL	100 mL
Working Solution 1	1000 ppb	5 ppb	500 μL	99.5 mL	100 mL
Working Solution 2	1000 ppb	1 ppb	100 μL	99.9 mL	100 mL
Working Solution 3	1 ppb	500 ppt	5.00 mL	5.00 mL	10 mL
Working Solution 4	1 ppb	400 ppt	4.00 mL	6.00 mL	10 mL
Working Solution 5	1 ppb	350 ppt	3.50 mL	6.50 mL	10 mL
Working Solution 6	1 ppb	300 ppt	3.00 mL	7.00 mL	10 mL
Working Solution 7	1 ppb	250 ppt	2.50 mL	7.50 mL	10 mL
Working Solution 8	1 ppb	200 ppt	2.00 mL	8.00 mL	10 mL
Working Solution 9	1 ppb	175 ppt	1.75 mL	8.25 mL	10 mL
Working Solution 10	1 ppb	150 ppt	1.50 mL	8.50 mL	10 mL
Working Solution 11	1 ppb	125 ppt	1.25 mL	8.75 mL	10 mL
Working Solution 12	1 ppb	100 ppt	1.00 mL	9.00 mL	10 mL
Working Solution 13	1 ppb	75 ppt	0.75 mL	9.25 mL	10 mL
Working Solution 14	1 ppb	50 ppt	0.50 mL	9.50 mL	10 mL
Working Solution 15	1 ppb	25 ppt	0.25 mL	9.75 mL	10 mL