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Direct aqueous injection of the fluoroacetate anion in potable water for analysis by liquid chromatography tandem mass-spectrometry

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Abstract

Sodium fluoroacetate or Compound 1080 is a rodenticide registered in the United States for use in livestock protection collars. The collars are employed to control predation on herd animals (i.e., killing of cattle by wolves or coyotes). Sodium fluoroacetate is acutely toxic to humans and has potential to cause mass casualties if used to intentionally contaminate water systems. The U.S. Environmental Protection Agency (EPA) is responsible for characterization and remediation if such an incident occurs in the civilian sector. In support of that goal, EPA has published the *Selected Analytical Methods for Remediation and Recovery* (SAM) document that provides sampling and analysis methods for many hazardous chemicals such as sodium fluoroacetate. Ideal SAM methods require limited sample preparation steps and utilize widely available equipment to ensure the ability for maximum laboratory participation in a large-scale response. The present paper describes a direct aqueous injection (DAI) method for liquid chromatography/tandem mass spectrometry (LC-MS/MS) analysis of the fluoroacetate anion (FAA) in potable water. Sample preservation and filtration are the only pre-processing steps required. FAA is chromatographically separated on an octylsilane (C8) reversed phase column. Separation is attributed to ion-exchange interactions. Electrospray ionization (ESI) in negative mode and detection by tandem mass spectrometry follow. FAA presence was confirmed by two fragment ions in the correct ratio, and use of a labeled standard allowed for quantitation by isotope dilution. FAA detection and quantitation limits were 0.4 µg/L and 2 µg/L, respectively. Four different drinking water utilities provided water samples from varying locations across the U.S. All the water samples were fortified with FAA and tested to evaluate analyte stability and the robustness of the method.

1.0 Introduction

Sodium fluoroacetate, or Compound 1080, is a restricted use rodenticide registered by the United States Environmental Protection Agency (EPA) for use in livestock protection collars. Predators attacking the livestock bite the collars, leading to ingestion of Compound 1080 and subsequent morbidity or mortality of the predator. It is also used in New Zealand for possum control by aerially deployed bait [1]. Sodium fluoroacetate is a potent poison to

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mammals with an LD₅₀ in humans of 0.7–2.1 mg/kg [2]. The sodium salt dissociates to the fluoroacetate anion (FAA, Figure 1) in water and is highly soluble. The abbreviation FAA will be used to encompass both the protonated and un-ionized forms in water. Previous studies in streams report stability of FAA for several days depending on the biological activity of the water [3].

Concern for sodium fluoroacetate as a potential threat to potable water sources has led to the need to develop a method for emergency response scenarios. EPA's *Selected Analytical Methods for Remediation and Recovery* (SAM) tool can assist emergency responders with a list of recommended methods that can be used for target analytes in environmental matrices [4]. The SAM program strives to provide analytical methods to facilitate quick characterization and recovery operations. Ideal SAM methods utilize widely available equipment and have minimal sample processing time to ensure feasibility of a large-scale response.

Traditional FAA methods require a derivatization step followed by gas chromatographic (GC) separation and analysis with sensitive detection limits [5, 6]. For example, Ozawa and Tsukioka report a sub-part per billion level detection limit in water [5]. However, the sample preparation procedures are extensive and time-consuming. To eliminate the derivatization step required for a GC separation, ion chromatography (IC) is an attractive alternative technique [7–9]. FAA is an ideal compound to analyze by IC because it has an estimated pK_a of 2.72 at 25 °C and would be ionized at typical potable water pH. IC coupled with suppressed conductivity detection has been successfully employed, but the detection limit was not as sensitive (21 µg/L) [7] and lacked the additional confirmation provided by mass spectrometry (MS). Another IC method used a post-column diversion step and the addition of acetonitrile to make the mobile phase compatible with MS. The authors achieved sensitivity comparable to GC, operating the MS in selected ion monitoring (SIM) mode, with large injection volumes and long run times [8, 9]. In recent years, liquid chromatography-tandem mass spectrometry (LC-MS/MS) instruments have become more common. Use of LC-MS/MS would avoid the necessity of a post-column addition step and provide additional confirmation using compound fragmentation. Recent efforts have developed LC-MS/MS analysis methods in both infant formula [10] and urine [11], yet both analyses require several pre-processing extraction steps to address complex matrix effects. Online extraction techniques are not yet widely used, making direct aqueous injection (DAI) the best option for high throughput analysis of water samples.

EPA regulates haloacetic acids containing chlorine or bromine as drinking water contaminants. These compounds are formed as byproducts of the disinfection process [12]. Multiple methods have been published for these chemicals in water and the techniques described may have application to FAA. Several methods suggest the use of ion-pairing reagents to alleviate complications associated with chromatography and sample pre-concentration steps [13, 14]. Other methods include DAI on either a reversed phase column [15] or a mixed mode column [16].

Adequate retention of FAA on the chromatography column can increase the ionization (i.e., the sensitivity) due to the increased organic solvent at the end of a reversed phase gradient

and facilitate separation from interfering sample components. The hydrophobic-subtraction (H-S) model notes five parameters of solute-column interactions that can describe retention [17–19]. Each parameter consists of the product of the column value and the analyte value. The five parameters associated with the column have been characterized for hundreds of columns and are publicly available at the United States Pharmacopeia website [20]. Viewing the separation in the context of these parameters may give insight into important column characteristics influencing the separation.

The present paper describes a DAI-LC-MS/MS method for the detection of FAA in drinking water. The procedure eliminates sample preparation steps that can be time-consuming and utilizes widely available equipment. We also discuss FAA retention in the context of the H-S model to identify the important separation parameters. FAA stability during storage and method robustness have been demonstrated by testing the method with water sources of varying characteristics.

2.0 Materials and Methods

2.1 Chemicals and Reagents

Sodium monofluoroacetate (FAA) and labeled sodium monofluoroacetate ($^{13}\text{C}_2$, 99%; $^2\text{D}_2$, 98%, FAA_L) were purchased from Cambridge Isotope Laboratories, Inc. (Andover, MA, USA). A secondary source standard of FAA was used for concentration verification and purchased from SPEX CertiPrep, LLC (Metuchen, NJ, USA). All solvents including water, methanol, and acetonitrile were LC-MS grade and purchased from Fisher Scientific (Pittsburgh, PA, USA). Mobile phase additives (acetic acid, formic acid, and ammonium formate) were Optima LC/MS grade and purchased from Fisher Scientific (Pittsburgh, PA, USA). Ammonium acetate was manufactured by MP Biomedicals (Santa Ana, CA, USA) with a listed purity of 98%. L (+)-ascorbic acid was purchased from Fisher Scientific as an Acros Organics chemical with 99% purity. Sodium omadine was purchased from Sigma Aldrich (St. Louis, MO, USA).

2.2 Water Samples

Four different water utilities (WS 1–4) across the USA each provided 500 mL of finished tap water. Water utilities were selected to obtain samples with widely varying water quality parameters (total organic carbon (TOC), conductivity, etc.) and treatment technologies (chlorination, chloramination, etc.). Reagent grade water (RW) was used as a blank matrix for calibration standards and Quality Assurance./Quality Control (QA/QC) samples. Water quality parameters were provided by the utility upon collection and are available in Table 1. Water samples were refrigerated (4 °C) upon arrival.

2.3 Sample Preservation and Preparation

Tap water samples were dechlorinated with L (+)-ascorbic acid to obtain a final concentration of 250 mg/L. Sodium omadine served as an anti-microbial at a final concentration of 64 mg/L. Samples were mixed by vortex and filtered through a 0.22- μm polyvinylidene fluoride (PVDF) syringe filter (Millex®-GV). A 990 μL aliquot was added to an LC vial to which 10 μL of the labeled internal standard (FAA_L) in water was added,

resulting in a final concentration of 25 µg/L of FAA_L. Calibration standards contained the same concentrations of preservatives as the samples but were not filtered. The effect of filtration on samples was evaluated in preserved RW by quantitating filtered and unfiltered aliquots.

2.4 LC-MS/MS Instrumental Conditions

2.4.1. Chromatographic Conditions—Chromatographic separation was achieved with an Accela LC System (Thermo Fisher Scientific, San Jose, CA, USA). The system consists of a quaternary 1250 series pump, a degasser, and an autosampler. A CTO-10AVP column oven (Shimadzu, Columbia, MD, USA) was also used. Chromatographic analysis was performed on an octylsilane (C8) column (Sunfire™, 5 µm, 4.6 mm × 150 mm, Waters, Milford, MA, USA). Separations were carried out at 37 °C, and the injection volume onto the column was 20 µL. Samples were held in the autosampler tray at 10 °C until analysis. The mobile phase composition was 0.005% acetic acid in both water (A) and acetonitrile (B). Gradient elution began with a flow rate of 0.5 mL/min of 95% A, held for 1 minute, then ramped over 10 minutes to 60% A. Between 11 and 14 minutes, A was decreased to 0% and held for 5 minutes. Between 19 and 20 min, A was ramped to 95%. At 21 min, the flow was increased to 1 mL/min and A decreased to 80%, after which (24 min) A ramped from 0% to 95% over 6 minutes. The column was re-equilibrated to initial conditions for 2.5 minutes. Total runtime including re-equilibration was 32 minutes (Supplementary Information, Table S1).

Capacity factor (k') was used to evaluate chromatographic retention. The parameter k' is defined as the relative difference between the analyte retention time (RT) and the retention time of a compound that has no affinity for the solid phase (i.e., elutes with the solvent front). A k' between 1–10 is considered acceptable.

2.4.2 Mass Spectrometry—The mass spectrometric analysis was performed on a TSQ Quantum Ultra Mass Spectrometer (Thermo Fisher Scientific, San Jose, CA, USA) in triple quadrupole MS/MS mode. Complete ionization of FAA was accomplished using electrospray ionization (ESI) source in negative ion mode. Nitrogen was purified (99.5%) by a nitrogen generator (Whatman, Haverhill, MA, USA) supplied with a Workplace Air System™ (Atlas Copco, Hanover, MD, USA) and used to supply the sheath gas, ion sweep gas, and the auxiliary gas pressure. Ultra-high purity argon was used as the collision gas. The capillary temperature was set at 275 °C. The spray voltage was –3300 V and the vaporizer temperature was 250 °C. The sheath gas pressure was optimized to an arbitrary value (ABV) of 50 (0–100), while the ion sweep and auxiliary gas pressures demonstrated optimum performance when not supplied. The tube lens offset was set to –90, and the collision gas pressure was optimum at 0.7 (0–5 ABV). Two transitions (one quantitation (QT) and one confirmation (CF)) were monitored simultaneously for both FAA and the labeled internal standard in multiple reaction monitoring (MRM) mode with a scan time of 0.1 seconds. Collision energy, precursor and product m/z values are listed in Table 2. The divert valve was set to waste for approximately the first and last ten minutes of analysis time. The flow was directed to the detector for 10 – 21 minutes. Both LC and MS were controlled by Xcalibur 2.2 (Thermo Fisher Scientific, San Jose, CA, USA).

2.5 Method validation

2.5.1 Limit of detection and quantification—FAA confirmation was accomplished by comparing the RT ($\pm 1.5\%$) with the FAA standard in preserved RW and the presence of the QT and CF ions in the correct ratio in accordance with previous practices and are presented in Table 3 [21]. A nine-point internal calibration curve was constructed with a concentration range of 1 $\mu\text{g/L}$ to 100 $\mu\text{g/L}$ (see Supplementary Information). A linear regression with 1/x weighting was applied and was considered acceptable with $R^2 = 0.99$. The detection limit was determined according to the updated EPA practices [22]. The reporting limit was calculated using seven 2 $\mu\text{g/L}$ standards as described in previous procedures and presented within the Supplementary Information [22, 23].

2.5.2 Accuracy and precision—WS 4 was selected at random and used for the inter- and intra-day accuracy and precision evaluation (Table 3). Samples were fortified with FAA at three different concentrations, 4, 12, and 40 $\mu\text{g/L}$. Five replicates at each concentration were injected once in a day (intra-day) and compared across five time-points in 11 days (inter-day). Accuracy was evaluated as compared to nominal value and precision by the relative standard deviation (RSD).

2.5.3 Stability—FAA stability was evaluated in five water types (RW, WS 1–4) at high and low concentrations (40 $\mu\text{g/L}$, 4 $\mu\text{g/L}$) over the course of 28 days. Different drinking water sources were selected to represent a diversity of characteristics (e.g., organic content, hardness, chlorination, chloramination) to determine the effect that specific water parameters may have on the method or potential FAA degradation. Forty-mL samples were prepared with the appropriate amount of L (+)-ascorbic acid and sodium omadine and fortified with FAA. Method blanks were prepared with the same reagents (without FAA) and analyzed in tandem with each batch of water samples. Samples were vigorously mixed by vortex and stored at $4 \pm 3^\circ\text{C}$. Subsamples ($n = 3$) were removed from the vials at each time point: 0, 1, 3, 7, 14, 21, and 28 days. Possible losses due to the subsampling method, which required repeated uncapping of the vials, was evaluated by comparison to stability measured in seven separate samples of WS 1 over the same 28-day time frame.

2.5.4 Carryover—Two carryover pathways were considered. First, the carryover of the analyte into the next sample (i.e., FAA signal in a blank after injection of a standard or sample $> 60 \mu\text{g/L}$). Second, carryover of concomitant water components that cause suppression in subsequent samples. Data were evaluated for both types with consideration of sample injection order and the presence of blanks.

3.0 Results and Discussion

3.1 Optimization of preservation and sample filtration

Drinking water analysis requires at least two pre-injection steps. The addition of sample preservatives (e.g., dechlorination and anti-microbial activity) to maintain the integrity of the analyte and filtration to protect instrumentation from small particulates. The use of DAI analysis eliminates additional sample preparation steps (e.g., solid phase extraction,

concentration, etc.) and minimizes sample preparation time to allow for rapid analysis of target analytes.

Drinking water disinfected by chlorination may require removal of residual chlorine to prevent degradation of target analytes. Once dechlorinated, samples also require an anti-microbial agent to prevent bacterial growth [24]. EPA Method 538 [25] utilizes ammonium acetate for dechlorination and sodium omadine for prevention of microbial growth. However, the presence of ammonium acetate caused the FAA peak to broaden (Supplementary Information, Figure S1a). L (+)-ascorbic acid is capable of removing residual chlorine [15] and worked well in combination with sodium omadine (Supplementary Information, Figure S1b).

Initial 28-day stability tests of FAA in tap water were conducted with and without preservative. The investigation did not show a substantial benefit for the addition of preservatives to the water samples (data not shown). Literature precedent suggests that FAA can be chlorine-resistant [26], and microbial degradation is the main breakdown pathway observed in streams [1, 3]. Disinfection may limit the microbial activity in drinking water, but microbes can re-enter drinking water through water system biofilms [27] and affect FAA. Storage temperature can also play a role in microbial degradation as warmer temperatures enhance biological degradation of FAA while cooler temperatures reduce it [3]. Therefore, sample storage at 4 °C is strongly encouraged, with only brief removal FAA samples for processing before being placed in the 10 °C sample tray. The low microbial activity of the drinking water combined with the cool storage temperatures minimize FAA loss. However, the presence of residual chlorine in samples may be detrimental to instrument hardware over the course of many analysis runs. To prevent such an occurrence, both a dechlorination agent (ascorbic acid) and an anti-microbial treatment (sodium omadine) were included in the method as sample preservatives. Since the addition of preservatives did not affect FAA signal or cause matrix effects, they were used for the tested water types.

Drinking water samples were filtered prior to injection on the LC-MS/MS. Initial tests demonstrated that syringe filters had no detrimental effect on FAA analysis in either high ($p = 0.29$) or low concentration samples ($p = 0.25$). Filtration tests results are provided in Supplementary Information, Table S2.

3.2 Optimization of Chromatography

Recent FAA methods have utilized a variety of chromatography columns. Bessaire et al. used a Waters BEH Amide column for FAA analysis in infant formula [9]. DAI is unsuited for hydrophilic interaction chromatography (HILIC) because injection of 100% water is incompatible with the organic initial conditions of a HILIC gradient. As noted by Chen et al. [16], sample dilution in acetonitrile would be required without additional sample processing steps, thereby reducing mass on column (i.e., detection level). Consequently, use of this column was not investigated further. Hamelin et al. [11] reported FAA analysis in urine using a Primesep B2 by SIELC, a mixed mode column. Samples were processed with solid phase extraction and then injected in acetonitrile. For our effort, optimal ionization of FAA was obtained using a low percentage (0.005%) of acetic acid in the mobile phase. Addition of acetate buffering caused a significant reduction in sensitivity. However, when using a

SIELC column, the RT of FAA injected in water was not consistent among water types without mobile phase buffering. The pH adjustment of the drinking water samples did not improve retention stability.

A C8 column was noted to have higher retention than an octadecyl (C18) column in a DAI method of haloacetic acids [15]. Retention behavior was attributed to the conformation of the alkyl chains during the highly aqueous portion of the gradient. The shorter C8 alkyl chains maintain a more trans conformation than the gauche conformation of the longer C18 ligands [15] and therefore are better at retaining small hydrophilic molecules like FAA. A Sunfire C8 column with the optimized mobile phase demonstrated FAA separation with a k' of approximately 5. RT was relatively resistant to changes in the gradient. A faster gradient only resulted in a minimal reduction of runtime but a greater decrease in sensitivity. As expected, sensitivity was optimal when elution coincided with the 100% acetonitrile portion of the gradient since acetonitrile enhances ionization.

If only considering the interaction of the column hydrophobicity (H) with fluoroacetate (~86% ionized at pH 3.5), the high retention on a reversed phase column is surprising. In the H-S model, the five column parameters that control retention are H, the dominant factor in many reversed phase separations, steric interactions (S), H-bonding of an analyte to (A) or from the column (B), and ionization (C) [17–19]. Of these, H usually dominates retention for neutral analytes, but for charged compounds, C can become equally or more important [17]. The C parameter has been quantified for multiple columns at both pH=2.8 and 7 and values are available in the online USP database [20]. A $C < 0$ indicates a positively charged column. Most positively charged columns are end-capped and made of high-quality Type B silica, minimizing any charge effects from unbonded silanols or metals. The positive charge has been attributed to residual reagent from the alkyl ligand bonding process [28–30]. The column used in the present study, the Sunfire C8, has a listed C of -0.278 at pH 2.8, making an ion exchange mechanism between FAA and the column possible at the method mobile phase pH of ~ 3.5. The C value at pH 7 is $C(7.0) = 0.006$.

We investigated the effect of column charge on the FAA separation by manipulating the ionic strength of the mobile phase, similar to Loeser and Drumm [28]. Addition of both acetate and formate buffers at varying concentrations (1 mM and 5 mM) served as a surrogate for ionic strength while pH was held constant. At higher buffer concentrations (5 mM), FAA eluted faster for both the acetate (pH = 4.5) and formate (pH = 3.5) buffers (Figure 2). This elution pattern supports an ion exchange retention mechanism, in which higher buffer concentrations increased electrostatic screening, thereby partially shielding the attraction between the positively charged solid phase and the negatively charged analyte, FAA, and thereby reducing retention. However, it is difficult to definitively attribute observations to the electrostatic screening effect alone because competition between FAA and the anionic buffer component for positively charged column sites could explain the reduced retention as well. However, both of these mechanisms rely on a positively charged column.

Conductivity, a commonly measured water quality parameter, is another surrogate used for ionic strength. Higher water sample conductivity predicted an earlier average retention time

in the WS samples ($\beta = -1.8$, $p = 0.03$). The raw data is graphed in Figure 3A, with regression parameters discussed in further detail in Supplementary Information, Discussion S1. The negative slope between conductivity and RT is also suggestive of electrostatic screening or competition. High concentrations of ions in the water, either screened the charge of the solid phase or competed with FAA for binding sites, hastened the elution of FAA. This effect was observed for the most conductive water source, WS 3. Overall, RT variation was minimal ($< \pm 1.5\%$), but deviations correlated with water conductivity. Our optimized mobile phase (0.005 % acetic acid) has no buffering ability to offset the effects of the injection of a high conductivity sample, which could affect method RT robustness. The method is designed for potable water, which generally has conductivities between 30–1500 $\mu\text{S}/\text{cm}$ [31], similar to the ranges of the water types tested.

3.3 Method Validation

EPA has established a chronic oral reference dose for sodium fluoroacetate [32]. A chronic oral reference dose is defined as an estimate of a daily chemical exposure to the human population (including sensitive subgroups) that will not cause deleterious effects over a lifetime. The EPA reference dose for sodium fluoroacetate is 0.00002 mg/kg/day [32], which would equate to a 70 kg person drinking 4 L of 0.4 $\mu\text{g}/\text{L}$ -contaminated water in a day. For tap water, the risk based EPA regional screening levels list a commensurate concentration of 0.4 $\mu\text{g}/\text{L}$ [33]. For comparison, the New Zealand Ministry of Health set a 2 $\mu\text{g}/\text{L}$ oral reference dose for streams that may later be sourced for human water supply [1]. In the present study, the FAA MDL was calculated to be 0.4 $\mu\text{g}/\text{L}$, and the reporting level of 2 $\mu\text{g}/\text{L}$ accounts for verification by a single laboratory. Further testing may result in lower reporting limits. Response linearity was observed over two orders of magnitude. The method was verified for treated tap water, but in the case of any contamination incident, a site-specific risk assessment would be required to set safety levels. The reported FAA MDL would likely be appropriate to support emergency response decisions.

RSD and relative error of replicates in WS 4 quantified the accuracy and precision of the method. The highest values were 10 % and 7 %, respectively, and were exhibited by the lowest FAA concentration, 4 $\mu\text{g}/\text{L}$ (Table 3). Generally, precision and accuracy were better in the inter-day averages, which could be attributed to the increased number of replicates. RT and ion ratio robustness were evaluated with these samples and results are presented in Table 3.

Over 28 days, FAA demonstrated stability with recoveries ranging between 70–120 % for all but two-time points. WS 3 (4 $\mu\text{g}/\text{L}$) had recoveries of 63% and 59% for days 3 and 28, respectively. However, a one-way ANOVA comparison of % recovery for all water sources was compared across the complete time period (including the reference, day 0), and the time factor was not significant ($p = 0.09$). It may be note-worthy that WS 3 had the highest conductance as well as the most pronounced matrix effects. For example, the average internal standard (IS) response in WS 3 had the largest deviation from the average calibration standard (RW) IS response, falling to –52 % relative difference. For comparison, the largest deviation IS for WS 4 (the 2nd highest conductivity) was –21 %. Use of internal standard calibration largely corrected for suppression but may have slightly increased the

error in the low concentration FAA samples. No difference in recovery was noted between repeated uncapping of a single vial method versus one vial per time point. Complete stability study data is available in Supplementary Information, Table S3.

Carryover of concomitant water components required the last 10 minutes of the FAA method to consist of a high flow gradient alternating between a high percentage of aqueous and organic solvent. If the column were not vigorously flushed following an injection of potable water, significant FAA suppression was observed in subsequent injections. After a blank injection, sensitivity would return to normal. This phenomenon was particularly acute in waters with high conductivities. However, the increased flushing eliminated the suppression. Second, FAA carryover was observed only after injection of FAA standards 100 µg/L (the high calibration standard). The syringe flushing program was adjusted, but residual FAA continued to carry over into the next analysis. Diluting high concentration samples or running additional blanks after highly concentrated samples and standards resolved carryover issues and is recommended. Highly concentrated samples may impact the number of samples that can be analyzed daily if carryover issues are present.

4.0 Conclusions

The potent poison, sodium fluoroacetate, dissociates to FAA and sodium cation in water. If a water contamination incident were to occur, a fast, robust analytical method would be needed to ensure water infrastructure safety. The present study describes a high throughput DAI method developed for the identification and quantification of FAA in drinking water. The method utilizes reversed phase LC chromatography and fragmentation by MS/MS for verification of analyte presence. The chromatographic separation relied on the ionization parameter of the column, C, described in the H-S subtraction model. Any decision on column substitution should consider the C parameter. The method was tested with water samples from four different water sources across the U.S., each with varying water quality parameters. Calibration linearity was observed over two orders of magnitude. The limit of detection was 8 pg on column, equivalent to 0.4 µg/L [33]. The advantages of this method are the elimination of pre-concentration or extraction steps, a method detection limit equivalent to conservative risk values, robust performance with drinking water samples with varying water quality, and utilization of widely available laboratory instrumentation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fluoroacetate anion

CAS: 513-62-2

$pK_a = 2.72$ at 25 °C

Figure 1.
Fluoroacetate anion (FAA) Structure.

Figure 2:

RT change of FAA (1 mg/mL) using different concentrations of buffers and the Sunfire™ C8 Column. A) 1 mg/L FAA – Extracted ion chromatogram (EIC) of 57.26 m/z with formate buffer (pH = 3.5) B) 1 mg/L FAA - EIC of 57.26 m/z with acetate buffer (pH = 4.5).

Figure 3: Sample conductivity effect on RT. A) Conductivity of water sample versus the average chromatographic RT. Error bars represent the standard deviation. B) EIC for FAA (57.26 m/z) in the four tested water types.

Table 1:

Water Quality Parameters from Water Utilities

	WS 1	WS 2	WS 3	WS 4
Date Collected	2/22/2017	2/23/2017	8/01/2017	8/10/2017
State Water Collected	Florida	Ohio	Wisconsin	Washington DC
pH	9.35	8.80	7.14	7.69
Turbidity¹	0.27 NTU	0.05 NTU	0.28 NTU	0 FAU
Conductivity(µS/cm)	348	287	1124	388
Alkalinity (mg/L)	76	63	346	65
Hardness (mg/L)	76	114	436	127
Free chlorine (mg/L)	0.03	1.3	0.4	0.07
Chloramine (mg/L)¹	NA	NA	NA	3.11 (mono)
Total Organic Carbon (mg/L)²	7.85	1.13	NA	NR

¹NTU=Nephelometric turbidity unit, FAU=Fornazin attenuation units²NA=not applicable³NR=not reported

Table 2:

MRM Transitions

Compound name	Monitored transition	Dwell Time (ms)	Collision Energy (eV)	Retention Time (min)	<i>I</i> Type
Fluoroacetate	77.1 > 57.3	100	14	19.4	QT
	77.1 > 33.0	100	14	19.4	CF
¹³ C ₂ , ² D ₂ Fluoroacetate	80.9 > 36.1	100	14	19.4	QT
	80.9 > 59.9	100	14	19.4	CF

*I*_{QT} = Quantification, m/z and CF = qualification, m/z

Table 3:

Accuracy and Precision Data for WS 4.

Concentration(µg/L)	Precision % RSD		Accuracy % Error		Validation Parameters ³		FAA confirmation ⁴	
	Intra-day (n=5)	Inter-day (n=25)	Intra-day (n=5) ¹	Inter-day (n=25)	MDL	MRL	CF/QT ratio ⁵	RT ⁵
4	10	8	7	-1	0.4	2	2.8 (0.41)	19.4 (0.03)
12	6	5	6	0.2			2.7 (0.15)	19.4 (0.02)
40	5	4	1.5	-0.6 ²			2.7 (0.16)	19.4 (0.02)

¹ Highest value of the 5 days analyzed

² One sample failed QC and was removed from analysis (n=24).

³ MDL = Method detection limit, MRL= Method reporting limit

⁴ Ion ratio and RT matching statistics across all samples, all days

⁵ Reported average and (standard deviation)