

Quantitation of Fluoroacetic Acid and Fluoroacetamide with Mass Spectrometric Detection

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INTRODUCTION

Fluoroacetate (Compound 1080) and fluoroacetamide are strong metabolic poisons commonly used as rodenticides and preadacides.^{1,2} Fluoroacetic acid is also an intermediate metabolite of many compounds, such as anticancer drugs 5-fluorouracil and fluoroethyl nitrosourea. Fluoroacetate is cheap and simple to synthesize, tasteless, and has a high solubility in water. Due to their high level of toxicity when ingested,³ and no known antidotes, Fluoroacetate and fluoroacetamide have been banned or restricted in many countries. The U.S. EPA has placed sodium fluoroacetate in Toxicity Category I, indicating the highest degree for acute oral toxicity. It is crucial to develop a method for analysis of both fluoroacetic acid and fluoroacetamide is shown here.

Analyses for fluoroacetate and fluoroacetamide have been performed previously using several methods, including gas chromatography,^{4,5} HPLC,^{6,7} and fluoride-electrode⁸ analytical techniques. Many of these techniques rely upon derivatization prior to analysis⁹⁻¹¹ and lack adequate sensitivity for low-level detection. Use of chromatographic separation with selected ion monitoring (SIM) mass spectrometric (MS) detection affords the opportunity to reliably identify these compounds at trace levels in water samples.

This study focuses on determination of fluoroacetate and fluoroacetamide without derivatization or sample pretreatment. An ion chromatographic (IC) method has been developed for fluoroacetate using an MS detector, and a reversed-phase liquid chromatographic separation method has been developed for determination of fluoroacetamide, retaining the MS as the preferred detector. A fortified drinking water sample was spiked with the analytes and injected directly. Monitoring of representative mass-to-charge ratios (m/z) in SIM mode on a single quadrupole instrument allowed for highly sensitive and selective analyses. Method performance parameters such as linearity, calibration range, precision, and accuracy, and detection limits are presented.

EXPERIMENTAL

Equipment

Dionex ICS-3000 RFIC™ System
DP Dual Pump
EG Eluent Generator (KOH)
CR-ATC Trap Cartridge
CD-2 Conductivity Detector
AS Autosampler
Dionex UltiMate® 3000 HPLC
LPG-3600 Gradient Pump
FLM-3100 Flow Manager
WPS-3000 Autosampler
UVD340U UV Detector
MSQ Plus™ Single Quadrupole Mass Spectrometer
AXP-MS Auxiliary Pump
Chromeleon® 6.8 SR10 Chromatography Data System Software

Chromatographic Conditions

Fluoroacetate Separation (IC System)

Columns: IonPac® AG24 guard column (2.1 × 50 mm)
IonPac AS24 analytical column (2.1 × 250 mm)

Mobile Phase: Electrolytically generated KOH gradient

Gradient:	Time (min)	KOH (mM)
	0	5
	20	5
	20.1	80
	30.1	80
	30.2	5
	37.2	5

Flow Rate: 0.25 mL/min

Column Temp.: 15 °C

Detector (CD-2) Temp.: 20 °C

Inj. Volume: 100 µL (full loop injection)

Detection: Suppressed conductivity: ASRS® 300, 2 mm, external water mode (0.5 mL/min)

MS: See MS Parameters for Fluoroacetate

A diverter valve was plumbed after the CD-2 detector to allow for other compounds to be directed away from the MS after the elution of the analytes of interest. A flow of 0.1 mL/min of CH₃CN was added via a low-volume mixing tee prior to the MS to aid with the thermally assisted pneumatic nebulization of the electrospray ionization (ESI) source.

Divert Valve (Eluent Flow)

Time (min)	Position
0	To waste
0.1	To MS
20	To waste

MS Parameters for Fluoroacetate

ESI: Negative ion

SIM Scan: -77.0 *m/z* with 0.7 *m/z* span, dwell time 1.0 s

N₂ Pressure: 80 psi

Probe Temp.: 450 °C

Needle Voltage: 2 kV

Cone Voltage: 45 V

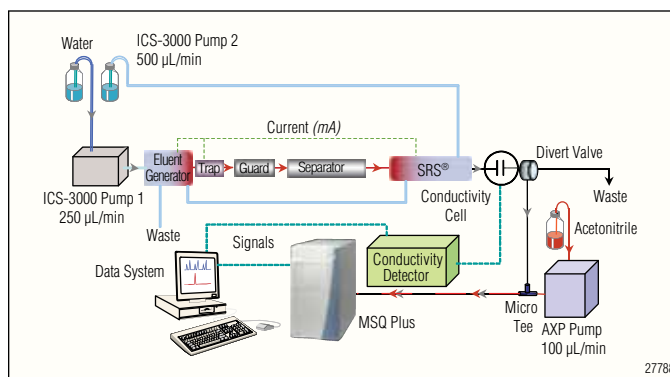


Figure 1. Schematic of an RFIC-MS system.

Fluoroacetamide Separation (HPLC System)

Column: Acclaim® RSLC PA2 (2.2 µm, 120 Å, 2.1 × 50 mm)

Mobile Phase: DI water, 100%, isocratic

Flow Rate: 0.25 mL/min

Column Temperature: 30 °C

Inj. Volume: 25 µL (50 µL loop, partial loop injection)

Detection: UV: Four single wavelength channels, 225, 250, 275, and 300 nm

MS: See MS Parameters for Fluoroacetamide table

MS Parameters for Fluoroacetamide

ESI: Positive ion

SIM Scan: 78.0 *m/z* with 0.5 *m/z* span, dwell time 0.8 second

N₂ pressure: 80 psi

Probe Temperature: 625 °C

Needle Voltage: 5 kV

Cone Voltage: 50 V

After flowing through the UV, the AXP Makeup Pump flow introduced an additional 0.15 mL/min of acetonitrile/0.1% formic acid to aid in volatilization and ionization in the ESI spray head of the MS.

Chemicals and Reagents

Sodium fluoroacetate (FA) was purchased from Sigma-Aldrich (CAS 62-74-8, Aldrich: 36755). Fluoroacetamide (FAA) was purchased from Sigma-Aldrich (CAS 640-19-7, Aldrich: 128341). Figure 2 shows the chemical structures.

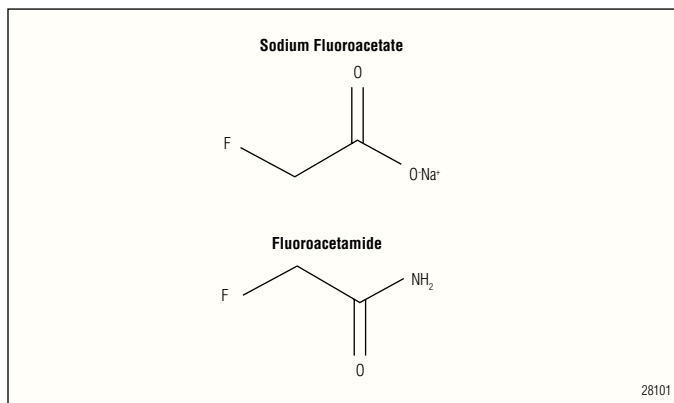


Figure 2. Structures of sodium fluoroacetate and fluoroacetamide.

Acetonitrile was obtained from Burdick & Jackson (HPLC grade, AH015-4). Deionized (DI) water was produced by a Millipore water station with 18.2 M Ω -cm resistance. Salts to make a fortified drinking water (DW) included 250 mg/L sodium chloride (NaCl, CAS 7647-14-5, J.T. Baker 4058-05), 250 mg/L sodium sulfate (Na₂SO₄, CAS 7757-82-6, EM Science SX0760-1), 150 mg/L sodium bicarbonate (NaHCO₃, CAS 144-55-8, EM Science SX0320-1) and 30 mg/L sodium nitrate (NaNO₃, CAS 7631-99-4, Aldrich 22,134-1).

A primary stock solution of FA was prepared at 1000 μ g/mL (ppm) in DI water. Separately, a primary stock solution of FAA was also prepared at 1000 μ g/mL (ppm) in DI water. Working stock solutions were prepared by diluting the primary stock solutions individually into DI and DW at 10 ppm and 100 ppb concentrations. These subsequently were used to prepare calibration standards and spiked DW samples.

Calibration

Fluoroacetate standards were prepared in clean (DI) water at 12 concentrations: 0.1, 0.2, 0.5, 1, 2, 5, 10, 20, 50, 100, 200, and 500 ppb. Full loop injections of 100 μ L yielded a total amount of 10 pg to 50 ng loaded on column (Figure 3).

Samples were prepared in fortified DW, spiked at the same 12 levels as the DI standards. To obtain an estimate of the limits of detection (LOD), a 2 ppb standard in DI water and a 2 ppb concentration in DW were each analyzed seven times.

Fluoroacetamide standards were prepared in DI water ranging in concentrations of 20, 50, 100, 200, 500, 1000, 5000, and 10,000 ppb. Partial loop injections of 25 μ L yielded a total amount from 500 pg to 250 ng loaded on column (Figure 4). LOD samples were prepared at 5 ppb in DI water and 20 ppb in synthetic DW.

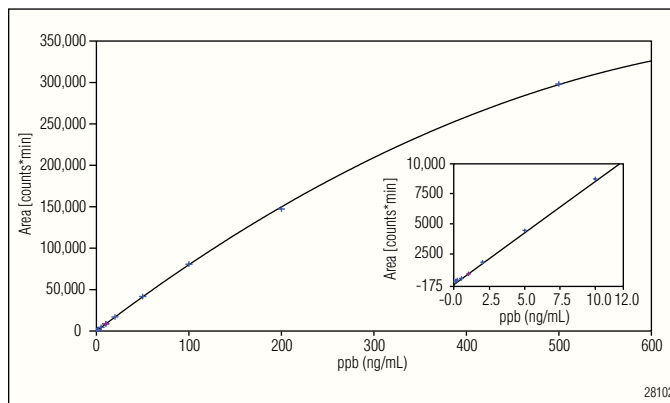


Figure 3. Calibration curve of fluoroacetate, 0.1–100 ppb in DI water.

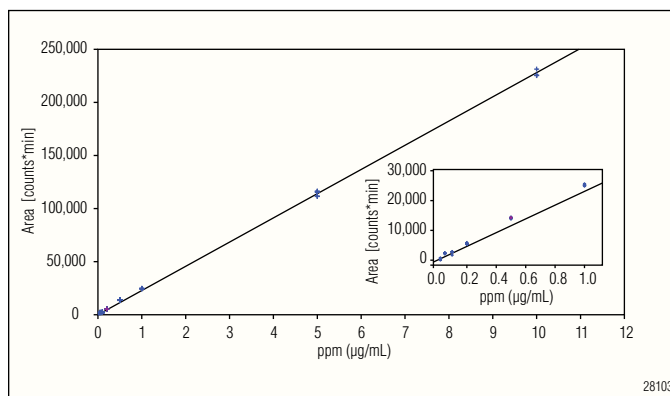


Figure 4. Calibration curve for fluoroacetamide 20 ppb–10 ppm.

RESULTS

Chromatography

Fluoroacetate was retained well on the IonPac AS24 column (figure 5). A retention factor (*k*) of 8 was observed, indicating sufficient retention to separate the target from matrix interferences. The IonPac AS24 column was chosen for its high capacity, allowing larger injection volumes and increasing the sensitivity of the analysis through chromatographic concentration of the target compound. While the analyte compound eluted at ~12.5 min, the total run time was 37.2 min to allow for any strongly retained species to be washed off the column with a high-strength eluent gradient before returning to starting conditions and equilibrating for the next sample injection. This improved method ruggedness, especially with the DW matrix.

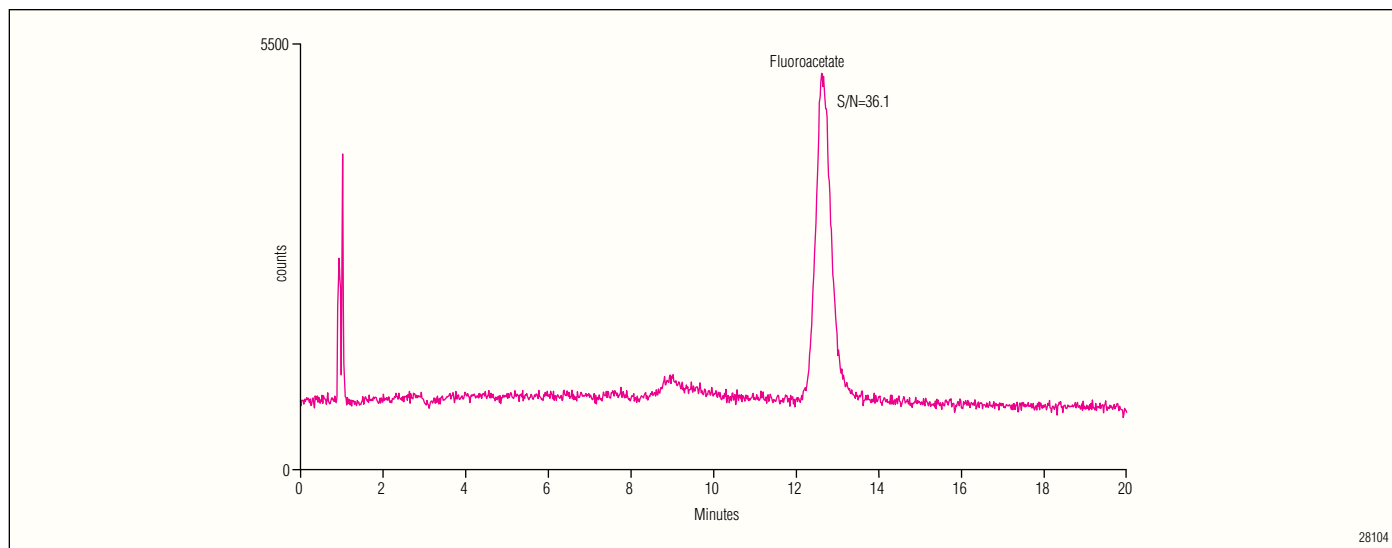


Figure 5. SIM chromatogram of $-77\ m/z$ fluoroacetate at 2 ppb in DI water.

The Acclaim RSLC PA2 column was chosen for the HPLC analysis of fluoroacetamide due to its compatibility with 100% aqueous conditions. The short (50 mm) column aided in quick run times, with the analyte eluting in just over 1 min (Figure 6). There were no peaks observed for the UV detector, thus confirming the necessity of using the mass spectrometer for identification and quantification.

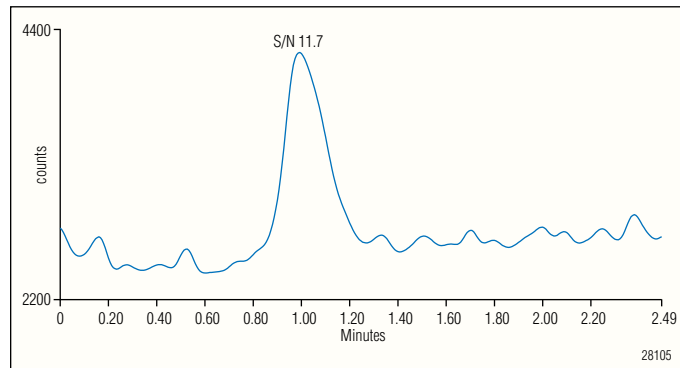


Figure 6. SIM chromatogram of $78\ m/z$, 5 ppb fluoroacetamide in DI water.

Mass Spectrometry

The aim of the study was to develop a selective and sensitive method for direct analysis of trace levels of fluoroacetate and fluoroacetamide in environmental water samples. MS was used in both cases. MS provides inherent selectivity based on m/z and operation in SIM mode provides increased sensitivity. Under IC conditions, the fluoroacetate showed a strong negative deprotonated molecular ion at $-77.0\ m/z$ in ESI mode. Analysis on the HPLC system of fluoroacetamide showed a positive protonated molecular ion at $78.0\ m/z$. The ionization parameters for each analyte were optimized independently, starting with varying cone voltage then needle voltage and finally probe temperature. Optimal parameters are recorded in the chromatographic conditions section. The scan dwell time was optimized to give good peak shape given the chromatographic width of the peak. Longer dwell times result in greater signal accumulation and better S/N but reduce the number of points across the chromatographic peak. Narrow chromatographic peaks require shorter dwell times to maintain good peak shape. It should be noted that optimal parameters for MS analysis are instrument and compound dependent, and any user wishing to repeat these experiments is advised to evaluate all acquisition parameters to determine optimal values for a different system and analytes.

Method Performance

Selectivity for fluoroacetate was established through the use of an SIM scan on the molecular ion, and when combined with chromatographic retention time ensured that the fluoroacetate was being accurately identified. Carryover was evaluated by injecting sample blanks (DI water) after a 500 ppb standard chromatographic run. No detectable peak was observed at the specific retention time. A quadratic curve best fit the calibration data as high concentrations contributed to saturation effects of the MS detector. Accuracy was calculated as observed amount/specified amount $\times 100\%$. Recovery accuracy in DW was within 10% across the concentrations from 0.5 ppb to 200 ppb, with only one exception (Figure 7 and Table 1).

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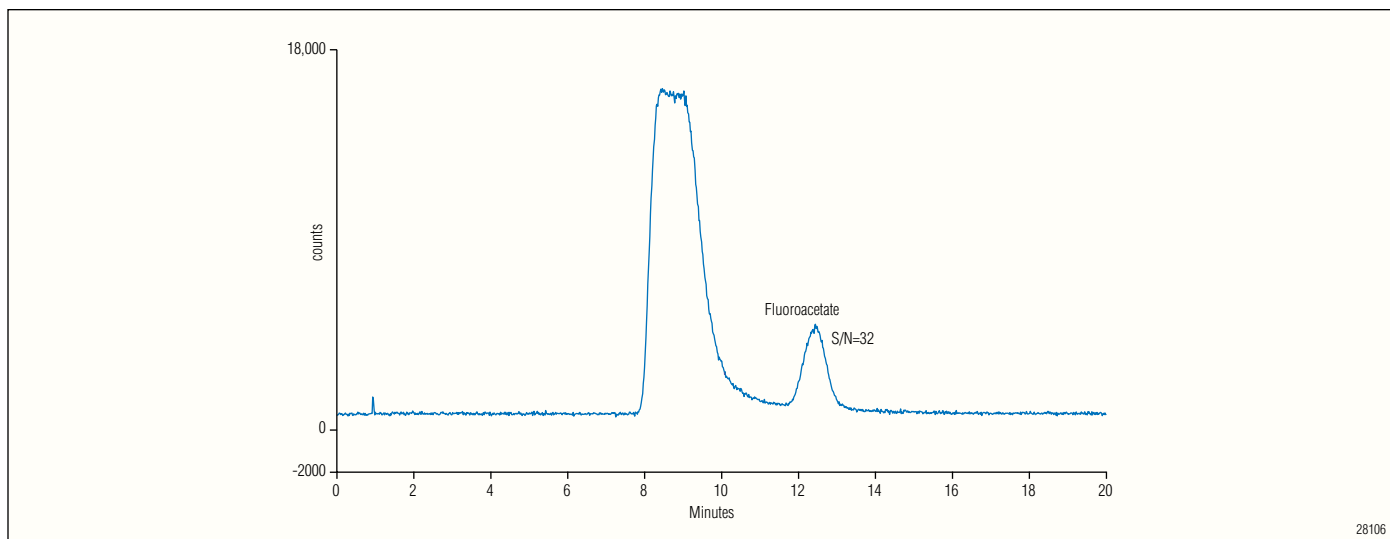


Figure 7. SIM chromatogram of -77 m/z, 2 ppb fluoroacetate in drinking water (DW).

Table 1. Fortified Drinking Water Response			
Concentration (ppb)	Average (n = 3)	RSD	Recovery (%)
0.10	ND	—	—
0.20	ND	—	—
0.50	0.47	12.52	93
1.00	0.92	12.53t	92
2.00	1.51	43.64	75
5.00	4.96	7.55	99
10.00	9.52	15.97	95
20.00	20.64	20.46	103
50.00	53.25	18.17	107
100.00	89.96	10.74	90
200.00	216.42*	12.25	108
500.00	395.89	6.68	79

*Average of two analyses

Run-to-run precision and accuracy was evaluated by seven replicate injections of a low-level standard at 2 ppb. Method detection limit (MDL) was estimated using the standard deviation obtained from the seven replicates using the following equation:

$$MDL = S \times t_{99\% \ n-1=6}$$

where S is the standard deviation and t is the Student's t at 99% confidence interval. Detection limits were calculated to be 0.3 ppb for fluoroacetate in DI water and 1.8 ppb in DW. This method illustrates that good separation can be achieved using the IonPac AS24 column, and when combined with the selectivity and sensitivity of the MSQ Plus mass spectrometer provides accurate identification and quantification of the compound.

Fluoroacetamide was accurately identified through the use of the SIM acquisition scan mode on the pseudomolecular ion in combination with the chromatographic retention time. Linearity of response across the calibration curve was determined using eight standards ranging from 20 ppb to 10 ppm, which resulted in a correlation coefficient (r²) value greater than 0.999 (Figure 4). The MDL was statistically calculated using the standard deviation obtained from seven replicate analyses using the same formula as above. Detection limits calculated for fluoroacetate were 2.4 ppb in DI water and 11.9 ppb in DW.

CONCLUSION

Methods for analysis of fluoroacetate and fluoroacetamide have been developed and details shown here. The use of the MSQ Plus provided molecular ion analyte selectivity, and the use of the SIM function achieved good low-level quantification in water matrices. This was accomplished with minimal sample preparation and achieved low ppb levels of quantification. Matrix effects and recovery were evaluated using a fortified water matrix and results show good precision and reproducibility.

REFERENCES:

1. Kalmbach, E.R. Ten-Eighty, a War-Produced Rodenticide. *Science*. **1945**, 102 (2644), 232–233.
2. Casarett and Doull's Toxicology. 2nd ed. Macmillan Publishing Co.: New York, 1980; p. 395.
3. Proudfoot, A.T.; Bradberry, S.M.; Vale, J.A. Sodium Fluoroacetate Poisoning. *Toxicol. Rev.* **2006**, 25 (4), 213–219.
4. Koryagina, N.L.; Savelieva, E.I.; et al. Determination of Fluoroacetic Acid in Water and Biological Samples by GC-FID and GC-MS in Combination with Solid-Phase Microextraction. *Anal. Bioanal. Chem.* **2006**, 386 (5), 1395–1400.
5. Cai, X.; Zhang, D.; Ju, H.; Wu, G.; Liu, X. Fast Detection of Fluoroacetamide in Body Fluid Using Gas Chromatography–Mass Spectrometry after Solid-Phase Microextraction *J. Chromatogr., B.* **2004**, 802 (2), 239–245.
6. Collins, D.M.; Fawcett, J.P.; Rammell, C.G. Determination of Sodium Fluoroacetate (Compound 1080) in Poison Baits by HPLC. *Bull. Environ. Contam. Toxicol.* **1981**, 26 (5), 669–673.
7. Ming, H.; Shen, W. A. Simple, Quick and Sensitive LC/MS/MS Method for Analysis of Virulent Raticide-Fluoroacetamide and Sodium Fluoroacetate. *Modern Scientific Instruments.* **2006**, 6, 114–115.
8. Peters, J.A.; Baxter, K.J. Analytical Determination of Compound 1080 (Sodium Fluoroacetate) Residues in Biological Materials. *Bull. Environ. Contam. Toxicol.* **1974**, 11 (2), 177–183.
9. Mori, M.; Nakajima, H.; Seto, Y. Determination of Fluoroacetate in Aqueous Samples by Headspace Gas Chromatography. *J. Chromatogr. A.* **1996**, 736, 229–234.
10. Okuno I.; Meeker, D.L. Gas-Liquid Chromatographic Determination of Sodium Fluoroacetate (Compound 1080). *J. Assoc. Off. Anal. Chem.* **1980**, 63 (1), 49–55.
11. Allender, W.J. Determination of Sodium Fluoroacetate (Compound 1080) in Biological Tissues. *J. Anal. Toxicol.* **1990**, 14 (1), 45–49.

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LPN 2839-01 06/11

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