Application Note AN-M-017

IC-MS/MS analysis of trifluoroacetic acid according to DIN 38407-53

TFA: a pervasive PFAS breakdown product of increasing concern

Trifluoroacetic acid (TFA) in surface and drinking water raises concerns due to its persistence, mobility, and limited mitigation options [1–3]. As a PFAS (per- and polyfluoroalkyl substance) breakdown product, TFA is primarily released from industrial sites, while secondary sources include degradation of compounds containing trifluoromethyl groups (e.g., certain pesticides and refrigerants) [4–6]. To ensure reliable monitoring, the German standardization body DIN has published a standard for TFA analysis using direct injection LC-MS/MS. This method quantifies TFA in the range of 0.1–3.0 µg/L and has been validated across 12 laboratories in Germany and Switzerland [7,8].

This Application Note describes the validated method setup using ion chromatography (IC) with subsequent mass detection with a triple quadrupole mass spectrometer.

Trifluoroacetic acid (TFA) is an ultra-short-chain PFAS (per- and polyfluoroalkyl substance) belonging to the most toxic group of perfluorooctanoic acids (PFOA) (Figure 1) [9]. Overall, TFA concentrations are significantly higher than those of other PFASs, as TFA is a breakdown product of those substances [3]. For example, TFA accounted for more than 90% of the measured total PFASs in various analyzed German drinking waters [9]. It has emerged as a ubiquitous and persistent substance with high mobility due to its lack of sorption, and raises several environmental and regulatory concerns [3–6,10]. TFA's ongoing emissions are causing irreversible concentration increases, for example, in rainwater, soils, and drinking water, but also in human serum, plants, and plant-based foods [3]. Measured median concentrations of TFA in precipitation reached 0.29 µg/L in the USA [11], 0.21 µg/L in Germany [1], and 0.70 µg/L in China [12]. Drinking water median TFA concentrations, ranging from 0.08 µg/L (USA) to 0.5 µg/L (Switzerland), and even up to 1.5 µg/L (Germany), were either similar to or higher than the proposed limits of total PFAS in drinking water (0.5 µg/L) in the EU Drinking Water Directive [9,13].

The thresholds for irreversible effects are unclear, but mammalian toxicity studies suggest TFA is harmful to the liver and reproduction as well [3].

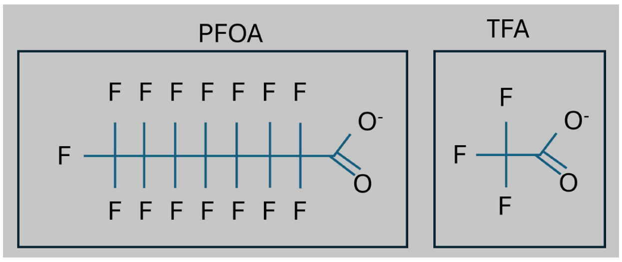

Figure 1. TFA and PFOA are types of polyfluorinated carboxylic acids. PFOA, referred to as «C8», contains eight carbon atoms, whereas TFA, the smallest in this subgroup, has two carbon atoms (adapted after Burtscher-Schaden et al. [9]).

As conventional water treatment technologies offer little to no removal efficiency for TFA, the risk of its accumulation is growing [3]. Consequently, environmental and health agencies are calling for tighter regulatory measures—such as restricting PFAS-containing pesticides and fluorinated gases—to limit further TFA formation [3,8,9].

In 2020, the German Federal Environment Agency (UBA) set a health guideline value of 60 µg/L for TFA in drinking water, but recommends not exceeding 10 µg/L as a precaution [14]. Other countries have set similar limits, such as 9 µg/L in Denmark and 2.2 µg/L in the Netherlands [15,16].

Recognizing the necessity for dependable monitoring, the German standardization organization DIN developed a standard for TFA analysis [7]. This method uses direct injection—liquid chromatography with tandem mass spectrometry (LC–MS/MS)—to quantify TFA in the 0.1–3.0 µg/L range and was validated by 12 labs in Germany and Switzerland [8].

Unlike most standards, DIN 38407-53 permits the use of various LC techniques, such as HPLC with HILIC, mixed-mode, and reversed-phase (RP) columns, as well as ion chromatographic approaches [7,8]. Metrohm enhanced and tailored the method for IC-MS/MS analysis to meet the requirements outlined in DIN 38407-53 [7,8]. This Application Note describes the method setup using a Metrohm ion chromatograph, a Metrosep A Supp 17 separation column, and mass detection with a 6475 triple quadrupole mass spectrometer from Agilent (*m/z* 113 and 69). The method passed all requirements and was successfully validated during the interlaboratory trial.

SAMPLE AND SAMPLE PREPARATION

Eight samples were included in the sample set: two groundwater samples, one rainwater sample, one drinking water sample, two surface water samples, and two spiked surface water samples with nominal concentrations of 1.18 µg/L and 2.87 µg/L TFA [8]. The samples were transported in a cooler and refrigerated at 4 °C prior to analysis. All samples were analyzed within seven days. No internal standard was used for the further fortification of samples and standards.

EXPERIMENTAL

Standards and samples were directly injected without further preparation steps (Figure 2). After separation on an anion-exchange column, TFA was detected with a 6475 triple quadrupole mass spectrometer from Agilent.

Sample and standard handling were performed using the fast 889 IC Sample Center – cool, increasing the stability of the liquids to be measured. A full-loop injection of 100 µL was executed. All measurements, including standards, blanks (0.1% methanol in ultrapure water), and samples, were performed in triplicate.

The separation of TFA from other anionic species was achieved on a Metrosep A Supp 17 – 150/4.0 column equipped with a Metrosep RP2 Guard/3.5 column to further improve the separation from anionic organic compounds. Isocratic conditions were used along with a carbonate eluent fortified with methanol (i.e., 5 mmol/L Na_2CO_3, 0.2 mmol/L $NaHCO_3$, and 10% methanol) to improve evaporation during electrospray ionization (ESI).

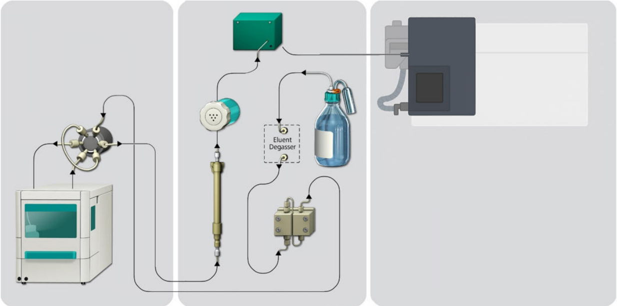

Figure 2. Setup for TFA analysis in water samples with an 889 IC Sample Center – cool (left) for sample introduction and sample cooling, a Metrohm 940 Professional IC, and a 6475 TQMS from Agilent Technologies (right). The conductivity detector is optional but highly recommended for method development, as it allows for effective monitoring of high concentrations of anionic compounds to prevent their entry into the ESI-MS/MS.

The IC was controlled with MagIC Net software which enables the detection of major anions, in addition to the target trifluoroacetic acid, through conductivity measurements. The MS signal was recorded using an Agilent 6475 TQ MS with a fragmentation voltage of 65.0 V to separate the ions at *m/z* 113 and 69. Data acquisition was performed using the Agilent MassHunter Workstation (LC/TQ) software. To synchronize the two devices, a remote box is mandatory.

The system was calibrated within the range of 0.2 to 4.0 µg/L (**Figure 3**). However, expanding the calibration range to 6.0 µg/L demonstrated adequate linearity in the mass spectrometry (MS) signal.

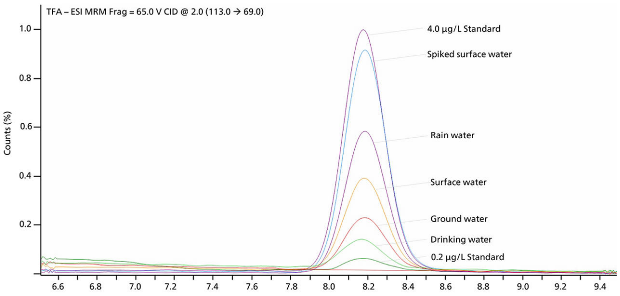

Figure 3. Chromatogram overlay of various water samples and TFA standards of 0.2 µg/L and 4.0 µg/L. The chromatograms were recorded in MS/MS mode using an Agilent 6475 TQ in dMRM mode with a fragmentation voltage of 65.0 V (collision induced dissociation, CID) and a mass fractionation of 113.0 to 69.0.

RESULTS

The calibration curves for the MS signal showed linear relationships, as required by the DIN method, with an R2 value of 0.9998. The calculated accuracy for the standards, based on the correlation between target concentration and measured concentration, ranged from 98.1% to 110.1%. The limits of detection (LOD) and limits of quantification (LOQ) were determined to be 0.017 µg/L and 0.062 µg/L, respectively, in accordance with DIN 32645 [17].

After triplicate analysis of the samples, the measured concentrations of TFA varied from 0.38 µg/L in drinking water to 1.93 µg/L in rainwater (Table 1). The spiked surface water samples had nominal concentrations of 1.18 µg/L and 2.87 µg/L. The validation test results were 1.28 ± 0.01 µg/L and 2.92 ± 0.01 µg/L, indicating recoveries of 109% and 102%, respectively.

The variation in triplicate measurements of the samples ranged from 0.3% to 2%. The retention time (RT) of TFA was on average 8.2 min, with a variation of less than 0.1%.

For the quality control (QC) standards at concentrations of 0.6 µg/L and 2.0 µg/L, the recovery rates across three replicate determinations within the sample series measurement were 100% and 99%, respectively.

All results met the requirements of the interlaboratory trial, which serves to validate the method.

Table 1. Summary of triplicate water analysis results for TFA

Sample	R1 [µg/L]	R2 [µg/L]	R3 [µg/L]	Mean [µg/L]	SD [µg/L]
Groundwater	0.7058	0.7031	0.6922	0.7004	0.01
Groundwater	0.7745	0.7728	0.7602	0.7692	0.01
Rainwater	1.9488	1.9382	1.9116	1.9329	0.02
Drinking water	0.3812	0.3796	0.3856	0.3821	0.00
Surface water	1.2145	1.2214	1.2165	1.2175	0.00
Surface water	0.9859	0.9649	0.9478	0.9662	0.02
Spiked surface water (1.18 µg/L TFA spike)	1.2930	1.2820	1.2732	1.2827	0.01
Spiked surface water (2.87 µg/L TFA spike)	2.9365	2.9203	2.9139	2.9236	0.01

CONCLUSION

The development and validation of DIN 38407-53 by IC-MS/MS marks a significant advancement in analytical methods for IC coupled with tandem mass spectrometry (MS/MS). Notably, IC-MS/MS exhibits distinct advantages when analyzing short-chain fluorinated compounds, such as TFA, due to its high matrix resistance and long column lifetimes. This makes IC-MS/MS a validated alternative approach to traditional HPLC methods.

The developed method meets the requirements of DIN 38407-53. Automated features such as inline dilution further enhance the practicality of using IC for trifluoroacetic acid analysis.

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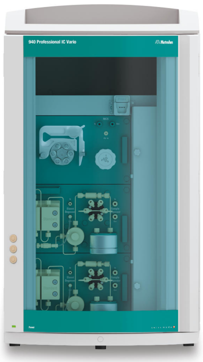
CONFIGURATION



940 Professional IC Vario TWO/SeS/PP

The 940 Professional IC Vario TWO/SeS/PP is the intelligent **two-channel** IC instrument with **sequential suppression** (one channel) and a **peristaltic pump** for suppressor regeneration. The instrument can be used with any separation and detection methods.

Typical areas of application:

- Standard instrument for parallel anion and cation determinations
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- Online monitoring for anions and cations

889 IC Sample Center – cool

The 889 IC Sample Center – cool is the appropriate automation solution when you have only a very small amount of sample. In comparison with the 889 IC Sample Center, it possesses in addition a cooling function and is thus the ideal sample changer for biochemically relevant or thermally unstable samples.

Metrosep A Supp 17 - 150/4.0

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Metrosep RP 2 Guard/3.5

The Metrosep RP 2 Guard/3.5 is a guard column for universal use. It reliably protects the analytical separation column against contaminations. It reliably protects the analytical separation column against contamination, removing the smallest particles, traces of iron oxide and bacteria. The Metrosep RP 2 Guard/3.5 helps to reduce costs; its filter disk can be replaced very easily.