

Confident quantification of per- and polyfluoroalkyl substances in water intended for human consumption

Using the QTRAP® 6500+ LC-MS/MS System and the SCIEX Triple Quad™ 7500 LC-MS/MS System – QTRAP® Ready

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Per- and polyfluoroalkyl substances (PFAS) are a family of thousands of synthetic compounds that are frequently found in the environment due to their widespread use in multiple industries. These compounds, which contain carbon-fluorine bonds, are incredibly resistant to breaking down. This means that they can accumulate within flora and fauna, and contaminate food and drinking water, leading to serious complications over time. Even if all manufacturing involving PFAS suddenly stopped, the existing environmental contamination would persist for decades to come. For this reason, it is paramount to limit the release of PFAS compounds into the environment. While some manufacturers have begun replacing PFAS compounds with either shorter-chain PFAS or non-fluorinated compounds, shorter-chain PFAS molecules have the potential for similar accumulation in the environment, food and drinking water.¹

In February 2020, the European Parliament and Council of the European Union released a new directive that recasts the limit of PFAS in drinking water to 0.5 µg/L for all PFAS compounds identified, and 0.1 µg/L for a subset of PFAS compounds that are deemed particularly concerning for humans (see Table 1 for the LOQ of each compound analyzed). EU member states can choose which limit to use, and they can also decide to implement both. The difference between the limits is dependent on a list of compounds stated within the directive. The 0.1 µg/L limit applies



to the compounds included in this list, which contain a perfluoroalkyl moiety with 3 or more carbons (i.e., –CnF2n–, n ≥ 3) or a perfluoroalkylether moiety with 2 or more carbons (i.e., –CnF2nOCmF2m–, n and m ≥ 1). The 0.5 µg/L limit applies to all PFAS compounds in totality.²

The requirements of this directive create the analytical challenge of achieving these newly set LOQs in drinking water without solid phase extraction (SPE) cleanup. This technical note demonstrates how these limits are easily achievable with a method that uses the QTRAP® 6500+ LC-MS/MS System for the analysis of all required PFAS compounds. In addition, it provides tips to help researchers achieve these limits and reduce common issues associated with the analysis without the use of specialized equipment or system modifications.

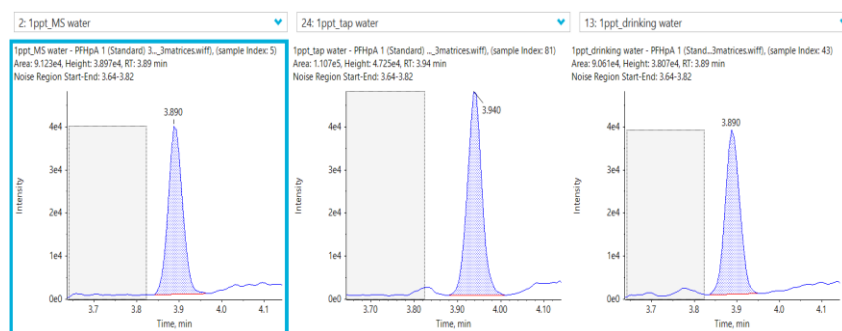


Figure 1. Extracted ion chromatograms (XICs) of PFHpA. XICs for PFHpA at the LOQ (0.001 µg/L) in 3 different water matrices: ultrapure HPLC-grade water (left), tap water (middle) and bottled mineral water (right).

The conditions used with the QTRAP 6500+ System were also tested on the SCIEX Triple Quad™ 7500 LC-MS/MS System – QTRAP® Ready to highlight the increased levels of sensitivity that can be achieved with this system.

See Figure 1 for example XICs from a PFAS compound at its LLOQ in 3 different water matrices.

Key features of the QTRAP 6500+ System for PFAS analysis

- LLOQ values down to 0.001 µg/L for the majority of the analyzed PFAS compounds
- The use of a 50 µL injection volume to meet the regulation requirement for drinking water (tap and bottled mineral water)
- Robust quantification with calibration curves for the vast majority of PFAS compounds between 0.001 and 1 µg/L
- No carryover or contamination in blank injections for analyzed PFAS compounds
- The use of a delay column to ensure that instrument contaminants are moved away from the analyte's retention time so that accurate quantification can be achieved

Methods

Standard preparation: Standards were provided by AGE (Luxembourg) and ordered from Wellington Laboratories (Guelph, Ontario).

Sample preparation: Calibration solutions were prepared by dilution of each water sample (ultrapure HPLC-grade water, tap water and bottled mineral water) with methanol at a 50:50 ratio.

Note: it is paramount for all solutions to be prepared and vialled in polypropylene vessels. This is necessary due to the potential adsorption of PFAS compounds to glass vessels, which decreases the sensitivity of analytes over time.⁵

Chromatography: Chromatographic separation was performed using the ExionLC™ AD System, which provides very low carryover and full UHPLC capabilities. The column used was a Phenomenex Luna Omega 3 µm PS C18 100 Å, 100 x 3.0 mm with a Phenomenex Luna Omega 1.6µm PS C18 100 Å, 50 x 2.1 mm delay column. Details of the chromatography are outlined in the supplementary information.³

Mass spectrometry: These experiments were performed using the QTRAP 6500+ System and the SCIEX 7500 System. The systems were operated in negative mode with electrospray ionization (ESI) using the Scheduled MRM™ Algorithm. Data was acquired using Analyst® Software. Details for the MS conditions are outlined in the supplementary information.³

Data processing: Data was processed using SCIEX OS Software.

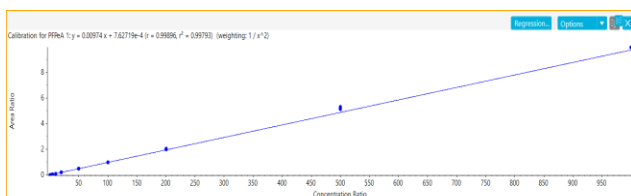
Table 1. Quantification of PFAS in various water matrices. LLOQ values for all PFAS compounds analyzed in ultrapure HPLC-grade water, tap water and bottled mineral water using the QTRAP 6500+ System. All LLOQ values provide an S/N (peak-to-peak) value above 10. A total of all PFAS compounds has been calculated for both the 0.1 µg/L and the 0.5 µg/L limits (bottom). All the compounds are relevant to the 0.5 µg/L limit. See Table 3 for a list detailed the full name of all compounds analyzed.

Compound	LLOQ (µg/L)		
	Ultrapure HPLC-grade water	Tap water	Bottled mineral water
<i>PFBA</i>	0.001	0.001	0.001
<i>PFPeA</i>	0.001	0.001	0.001
<i>L-PFBS</i>	0.001	0.001	0.001
<i>PFHxA</i>	0.001	0.001	0.001
<i>L-PFHeS</i>	0.001	0.001	0.001
<i>PFHpA</i>	0.001	0.001	0.001
<i>L-PFHxS</i>	0.001	0.001	0.001
<i>PFOA**</i>	0.001	0.001	0.001
<i>L-PFHpS</i>	0.001	0.001	0.001
<i>PFNA</i>	0.001	0.001	0.001
<i>L-PFOS</i>	0.001	0.001	0.001
<i>PFDA</i>	0.001	0.001	0.001
<i>L-PFNS</i>	0.001	0.001	0.001
<i>PFUdA</i>	0.001	0.001	0.001
<i>L-PFDS</i>	0.001	0.001	0.001
<i>PFDoA</i>	0.001	0.001	0.001
<i>PFTrDA</i>	0.001	0.010	0.005
<i>L-PFDoS</i>	0.001	0.001	0.001
<i>L-PFUdS***</i>	0.001	0.001	0.001
<i>L-PFTrDS***</i>	0.001	0.001	0.001
<i>PFTeDA*</i>	0.001	0.010	0.005
<i>PFHxDA*</i>	0.001	0.020	0.020
<i>PFODA*</i>	0.050	0.200	0.100
Total: 0.1 µg/L limit	0.020	0.029	0.024
Total: 0.5 µg/L limit	0.072	0.259	0.149

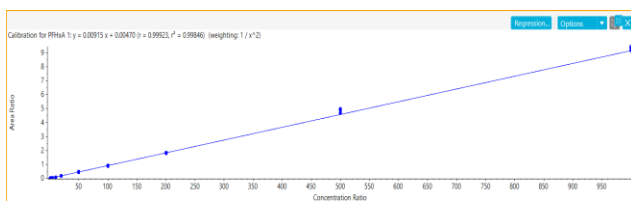
* Compounds only associated with the 0.5 µg/L regulation

** The LLOQ values associated with PFOA are dependent on a clean blank injection being achieved (in some instances, it has been noted that PFOA can provide a significant peak in the blank, which can impact the compound's LLOQ)

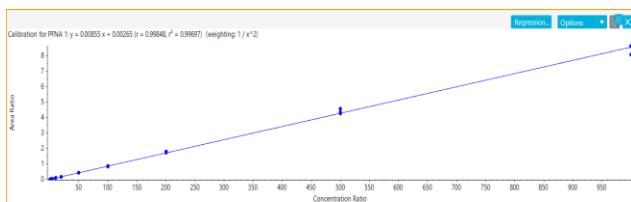
*** See the section "Recently regulated PFAS compounds" for more information on L-PFUdS and L-PFTrDS



Row	Component Name	Actual Concentration	Num. Values	Mean	Standard Deviation	Percent CV	Accuracy	Value #1	Value #2	Value #3
1	PFPeA.1	1.00	3 of 3	1.03	0.03	2.45	102.99	1.02	1.01	1.06
2	PFPeA.1	2.00	3 of 3	1.91	0.10	5.04	95.36	1.89	1.82	2.01
3	PFPeA.1	5.00	3 of 3	4.97	0.12	2.43	99.47	4.90	4.91	5.11
4	PFPeA.1	10.00	3 of 3	9.89	0.28	2.91	96.93	9.63	10.00	9.45
5	PFPeA.1	20.00	3 of 3	19.23	0.28	1.46	96.17	19.32	19.92	19.46
6	PFPeA.1	50.00	3 of 3	49.03	0.95	1.93	98.07	50.07	48.81	48.21
7	PFPeA.1	100.00	3 of 3	96.56	0.16	0.16	96.56	99.72	96.39	95.55
8	PFPeA.1	200.00	3 of 3	206.22	1.37	0.66	103.11	207.08	204.64	206.94
9	PFPeA.1	500.00	3 of 3	532.56	9.02	1.69	106.51	524.95	539.21	542.52
10	PFPeA.1	1000.00	3 of 3	1019.51	1.05	0.10	101.95	1019.37	1020.62	1018.54



Row	Component Name	Actual Concentration	Num. Values	Mean	Standard Deviation	Percent CV	Accuracy	Value #1	Value #2	Value #3
1	L-PFPeS.1	1.00	3 of 3	1.01	0.03	2.97	100.85	0.97	1.02	1.02
2	L-PFPeS.1	2.00	3 of 3	1.98	0.07	3.54	99.00	1.91	1.97	2.05
3	L-PFPeS.1	5.00	3 of 3	5.11	0.16	3.09	102.26	4.94	5.14	5.25
4	L-PFPeS.1	10.00	3 of 3	9.53	0.04	0.46	95.25	9.48	9.55	9.55
5	L-PFPeS.1	20.00	3 of 3	19.81	0.39	1.96	99.05	19.42	19.82	20.19
6	L-PFPeS.1	50.00	3 of 3	49.26	0.82	1.67	98.51	48.40	50.04	49.33
7	L-PFPeS.1	100.00	3 of 3	98.77	2.41	2.44	98.77	99.33	96.14	100.85
8	L-PFPeS.1	200.00	3 of 3	197.71	1.88	0.95	98.85	195.96	197.86	199.31
9	L-PFPeS.1	500.00	3 of 3	529.63	15.93	3.01	105.93	532.21	540.46	512.21
10	L-PFPeS.1	1000.00	3 of 3	1018.08	14.27	1.41	101.81	1022.28	1021.57	1020.37



Row	Component Name	Actual Concentration	Num. Values	Mean	Standard Deviation	Percent CV	Accuracy	Value #1	Value #2	Value #3
1	PFNA.1	1.00	3 of 3	0.99	0.12	12.31	98.57	0.92	0.91	1.13
2	PFNA.1	2.00	3 of 3	2.09	0.16	7.68	104.52	2.27	1.98	2.01
3	PFNA.1	5.00	3 of 3	4.85	0.05	1.07	96.93	4.83	4.91	4.81
4	PFNA.1	10.00	3 of 3	9.85	0.09	0.97	98.43	9.75	9.92	9.86
5	PFNA.1	20.00	3 of 3	20.31	0.36	1.79	101.57	20.09	20.12	20.73
6	PFNA.1	50.00	3 of 3	49.04	0.60	1.22	98.09	48.64	48.73	48.76
7	PFNA.1	100.00	3 of 3	96.55	1.01	1.03	96.55	96.82	97.40	99.33
8	PFNA.1	200.00	3 of 3	204.27	3.82	1.87	102.13	201.60	208.64	202.56
9	PFNA.1	500.00	3 of 3	513.99	18.36	3.57	102.80	500.55	514.91	506.50
10	PFNA.1	1000.00	3 of 3	983.89	35.87	3.64	98.39	1003.74	944.59	1000.32

Figure 4. Example calibration curves in HPLC grade water. Calibration curves of PFPeA (top), L-PFPeS (middle) and PFNA (bottom) spanning the range of 0.001–1 µg/L with accuracy values at each level between 80% and 120%, and %CV (%RSD) values below 10%.

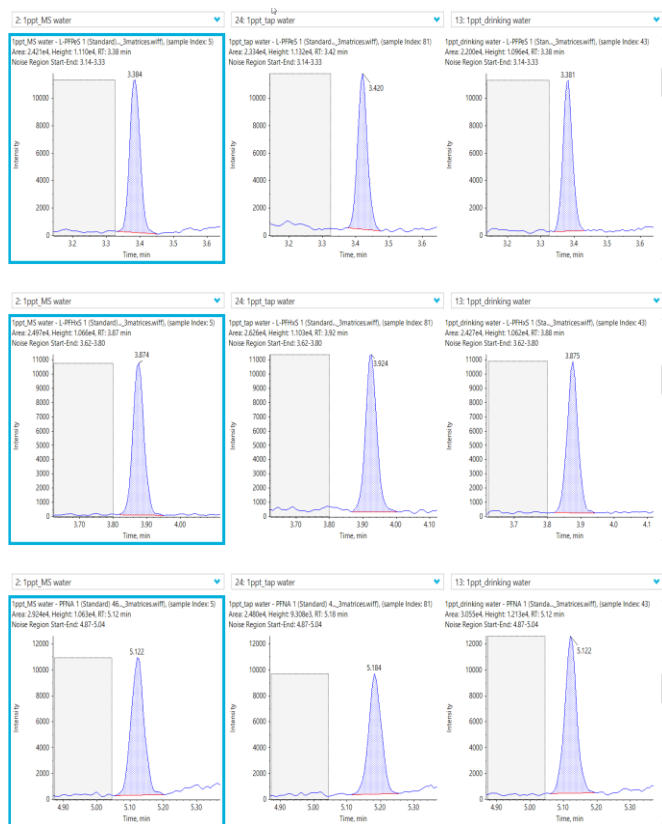


Figure 5. XICs for 3 representative PFAS compounds at LLOQs. XICs for L-PFPeA (top), L-PFPeS (middle) and PFNA (bottom) at an LLOQ of 0.001 µg/L in ultrapure HPLC-grade water (left), tap water (middle) and drinking water (right).

PFAS stability in glass

A significant issue with PFAS analysis is that long-chain PFAS compounds have low solubility in water samples and tend to stick to the surface of sample containers. This effect increases with chain length, and this is the main reason that polypropylene material is recommended for storage of samples and standards, and that methanol is added during sample preparation. To evaluate this effect, samples were stored in glass vials and the peak areas for all PFAS compounds were analyzed across 34 hours. The example for PFTeDA is shown in Figure 6, and the results across all PFAS are summarized in Table 2. Of the 21 compounds analyzed, 8 were susceptible to this issue in ultrapure HPLC-grade water and 11 were seen to decrease in both tap water and mineral water matrices. To account for this decrease over long analysis times, it is important to either use polypropylene vials during storage and analysis (Figure 7) or utilize deuterated internal standards to mitigate any change in signal intensity.

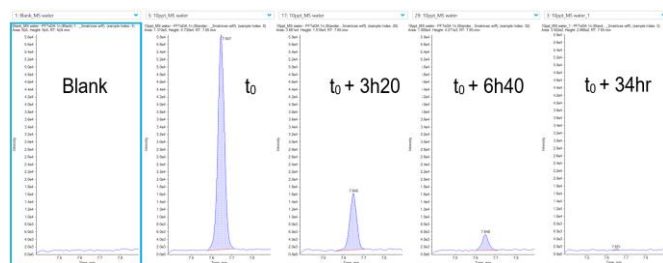


Figure 6. Stability assessment of PFTeDA in glass vials over time. XICs showing the sensitivity decrease of PFTeDA over a 34-hour period when using glass vials, which highlights the issue observed for many of the analyzed PFAS compounds. This demonstrates the importance of understanding this aspect of the analysis and accounting for it accordingly.

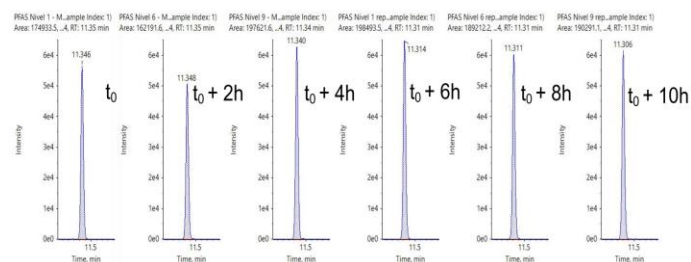


Figure 7. Stability assessment of MPFDoA in polypropylene vials over time. XICs of MPFDoA (an internal standard of PFDaA) over a 10-hour period when using polypropylene vials. The sensitivity of PFDaA was shown to decrease over time in glass vials (Table 2). This highlights the stability of the peak area and height when using polypropylene vial material. The above XICs show the internal standard measured from standard solutions and drinking water samples from across the analysis, showing its consistency over time even in different solutions and matrices.

Workflow points to consider

Using a delay column

In previous PFAS analyses, contamination has been observed when using an analytical column alone. This contamination appears to originate from the LC system itself, which creates the analytical challenge of separating the contamination from the analyte of interest. To address this challenge, a pre-column or delay column has been used to ensure the observed contamination is well separated from the analyte. The column chosen for this was similar to the analytical column and utilized the same stationary phase.

Table 2. Effect of glass vials on PFAS stability. The compounds here were found to be susceptible to a sensitivity decrease over 34 hours when using glass vials. The decrease is more pronounced for longer-chain PFAS compounds and is unaffected by the water analyzed. This demonstrates the importance of using polypropylene vials to ameliorate this effect.

Compound	Sensitivity decrease observed (Y/N)		
	Ultrapure HPLC-grade water	Tap water	Bottled mineral water
PFBA	N	N	N
PFPeA	N	N	N
L-PFBS	N	N	N
PFHxA	N	N	N
L-PFHeS	N	N	N
PFHpA	N	N	N
L-PFHxS	N	N	N
PFOA	N	N	N
L-PFHpS	N	N	N
PFNA	N	Y	Y
L-PFOS	N	Y	Y
PFDA	N	Y	Y
L-PFNS	N	Y	Y
PFUdA	Y	Y	Y
L-PFDS	Y	Y	Y
PFDaA	Y	Y	Y
PFTrDA	Y	Y	Y
L-PFDoS	Y	Y	Y
PFTeDA	Y	Y	Y
PFHxDA	Y	Y	Y
PFODA	Y	Y	Y

Reducing injection volume

With past methods that used LC-MS/MS to quantify PFAS compounds in water, it was common to use large injection volumes—possibly even injecting multiple milliliters of sample and performing a trap-and-elute workflow to concentrate this volume and reduce peak broadening. However, this analysis leveraged the high sensitivity of the QTRAP 6500+ System, which enabled the use of a 50 μ L injection volume.

Therefore, there is no need for any specialized equipment, such as a CTC autosampler, or for any significant modification to the UHPLC system, other than using a 50–100 μ L sample loop. Another benefit of using a reduced injection volume is a reduction in the previously observed issues, such as a high background and contamination, which leads to a more robust and easier-to-implement workflow.

Reducing blank contamination

Due to the widespread use of PFAS compounds, it is difficult to achieve a clean blank injection because of the prevalence of contamination that usually originates from the analytical instrumentation itself. This issue is mitigated by the use of a delay column, which means that a clean blank was achieved for all 23 analyzed compounds. This is still an issue to consider when performing PFAS analysis, however.

Recently regulated PFAS compounds

The new EU drinking water directive (released in February 2020) included 2 new PFAS compounds of concern: L-PFUdS and L-PFTrDS. At the time of the directive's release, the sourcing of these compounds proved to be particularly challenging, and for this reason, they were not included in the bulk of the analysis performed here. However, in late 2020, it became clear that these compounds were now commercially available (Wellington Laboratories, Guelph, Ontario),⁴ and so testing was performed to assess their LLOQ (Table 1).

Additional analysis performed on the SCIEX 7500 System

Due to sensitivity being a significant challenge when performing PFAS analysis, testing was also performed on the SCIEX 7500 System, which is the most sensitive triple quadrupole MS system in the SCIEX portfolio. All 20 compounds within the 0.1 μ g/L regulation were analyzed, with 15 out of 20 analytes providing improved LLOQ values, 3 providing a comparable LLOQ and 2 having a higher LLOQ due to unexpected interferences, which were thought to be caused by contamination within the water used or related to chromatography (data not shown). Figure 8 and Figure 9 show example results from the calibration curve measured for L-PFOS on the SCIEX 7500 System.

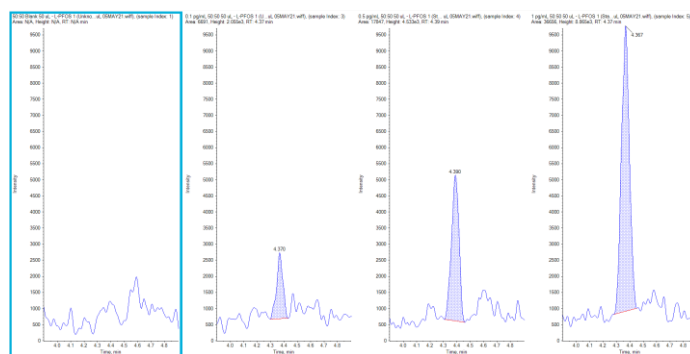


Figure 8. XICs of L-PFOS analyzed on the SCIEX 7500 System. The XICs for L-PFOS are (from left to right) 0.0001 μ g/L, 0.0005 μ g/L and 0.001 μ g/L. All analysis has been performed using a diluent of a 50:50 ultrapure HPLC-grade water and methanol mix.

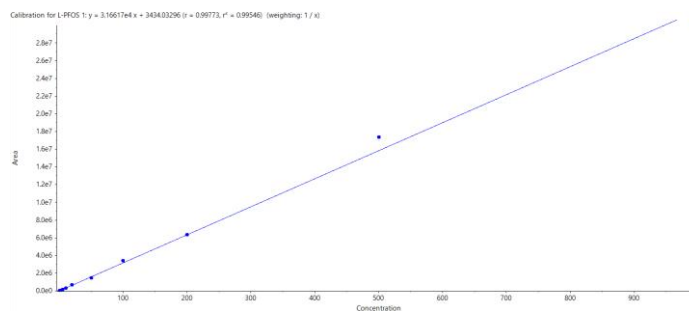


Figure 9. Calibration curve of L-PFOS on the SCIEX 7500 System. Calibration curve for data depicted in Figure 8 showing the linearity of the data from 0.0001–1 μ g/L, highlighting both the sensitivity of the system and the linear dynamic range that has been achieved ($r = > 0.99$).

Conclusions

To summarize, an LC-MRM method using a smaller volume direct injection approach has been demonstrated to be sensitive enough to easily meet the current recommended limits set by the European authorities for drinking water. The generated results highlight the accuracy, robustness and precision of the method. Several analytical challenges have been well documented for PFAS analysis in the past, such as blank contamination, compound stability and the need to inject large volumes of samples. This method has successfully addressed these problems, ensuring that it can be much more easily implemented compared with previous methods. It also does not require the use of specialized equipment or extensive modifications to the LC system. In addition, data has been generated using this method on the SCIEX 7500 System to highlight the advantages of increased sensitivity.

References

1. European Chemicals Agency (ECHA), [Perfluoroalkyl chemicals \(PFAS\)](#).
2. Council of the European Union, Proposal for a Directive of the European Parliament and of the Council on the quality of water intended for human consumption, [February 2020](#).
3. Download the [supplementary information](#).
4. Wellington Laboratories, New products: Native Certified Reference Standards for L-PFUdS and L-PFTrDS, [November 2020](#).
5. US Environmental Protection Agency, Determination of Selected Perfluorinated Alkyl Acids in Drinking Water by Solid Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS), Method 537 Rev 1, November 2008 ([revised October 2015](#)).

Table 3. A list of all compounds analyzed.

Compound name	Acronym
<i>Perfluorobutanoic acid</i>	PFBA
<i>Perfluoropentanoic acid</i>	PFPeA
<i>Perfluorohexanoic acid</i>	PFHxA
<i>Perfluoroheptanoic acid</i>	PFHpA
<i>Perfluorooctanoic acid</i>	PFOA
<i>Perfluorononanoic acid</i>	PFNA
<i>Perfluorodecanoic acid</i>	PFDA
<i>Perfluoroundecanoic acid</i>	PFUdA
<i>Perfluorododecanoic acid</i>	PFDoA
<i>Perfluorotridecanoic acid</i>	PFTTrDA
<i>Perfluorobutane sulfonic acid</i>	L-PFBS
<i>Perfluoropentane sulfonic acid</i>	L-PFPeS
<i>Perfluorohexane sulfonic acid</i>	L-PFHxS
<i>Perfluoroheptane sulfonic acid</i>	L-PFHpS
<i>Perfluorooctane sulfonic acid</i>	L-PFOS
<i>Perfluorononane sulfonic acid</i>	L-PFNS
<i>Perfluorodecane sulfonic acid</i>	L-PFDS
<i>Perfluorododecane sulfonic acid</i>	L-PFDoS
<i>Perfluorotetradecanoic acid</i>	PFTeDA
<i>Perfluorohexadecanoic acid</i>	PFHxDA
<i>Perfluorooctadecanoic acid</i>	PFODA
<i>Perfluoroundecane sulfonic acid</i>	L-PFUdS
<i>Perfluorotridecane sulfonic acid</i>	L-PFTTrDS

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