Editorial comments:

Changes to be made by the author(s) regarding the manuscript:

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.

2. Please add more details to your protocol steps. There should be enough detail in each step to supplement the actions seen in the video so that viewers can easily replicate the protocol. Please ensure you answer the “how” question, i.e., how is the step performed? Alternatively, add references to published material specifying how to perform the protocol action. See examples below.

3. 1.1.2: Please specify the PFAS standard used.

Methods have been altered to reference Wellington PFC Mix A (or custom mixture)

4. 2.2.1: Please specify the PFAS standards and the range of concentrations selected.

Methods have been altered to reference Wellington PFC Mix A (or custom mixture) and specify the points for an example calibration range (0-1000ng/L)

5. 2.3.1: Please specify the stable isotope labeled PFAS standard used.

Methods have been altered to reference Wellington MPFC Mix A (or custom mixture of SIL standards)

6. 2.4.1: Please spell out GF/A. What is the pore size of the filter?

Details of the Whatman GF/A filters have been moved from materials to methods

7. 2.6.3: Please provide more details about how to analyze the samples using an LC-MS/MS method.

A sample worklist, LC, and MS settings for comparison have been added to the manuscript. The manuscript assumes basic user competence at operation of an LC-MS/MS per the manufacturer instructions.

8. 2.6.5: Please describe how to obtain the IS corrected responses.

Manuscript now refers to the Raw/ISTD response ratio when generating calibration curves.

9. 2.7.3: Please specify the LC-gradient used.

LC gradient table now properly included.

10. 2.8 and substeps: Software must have a GUI (graphical user interface) and software steps must be more explicitly explained ('click', 'select', etc.). Please add more specific details (e.g. button clicks for software actions, numerical values for settings, etc.) to your protocol steps.

In depth substeps for the use of Agilent vendor software for data analysis are now included for reference.

11. Please combine some of the shorter Protocol steps so that individual steps contain 2-3 actions and maximum of 4 sentences per step.

12. After you have made all the recommended changes to your protocol (listed above), please highlight 2.75 pages or less of the Protocol (including headings and spacing) that identifies the essential steps of the protocol for the video, i.e., the steps that should be visualized to tell the most cohesive story of the Protocol.

13. Please highlight complete sentences (not parts of sentences). Please ensure that the highlighted part of the step includes at least one action that is written in imperative tense.

14. Please include all relevant details that are required to perform the step in the highlighting. For example: If step 2.5 is highlighted for filming and the details of how to perform the step are given in steps 2.5.1 and 2.5.2, then the sub-steps where the details are provided must be highlighted.

15. Please number the figures in the sequence in which you refer to them in the manuscript text. Currently Figure 6 (line 290) is introduced before all other figures.

Figures have been relabeled as appropriate

16. Tables 1-3: These tables are missing from the current submission. Please upload each Table individually to your Editorial Manager account as an .xls or .xlsx file.

Tables are now properly included

17. Figure 2: Please describe the left, middle and right panels in more detail.

Caption updated

18. Figures 3 and 4: Please add the y-axes. Please describe what different colors represent in the figure legend of Figure 4.

Y-axes are normalized and arbitrary, but now included. Colors in figure 4 are also arbitrary, but now described in the figure caption.

19. Figure 5: Please define RT in the figure legend.

RT now correctly referred to as retention time in the figure legend.

Reviewers' comments:

Reviewer #1:

Manuscript Summary:

The manuscript presents a useful sample preparation and analytical procedure capable of performing quantitation of known presence of polyfluorinated organic compounds as well as semi-quantitation of unknown fluorinated compounds. The targeted quantification method appears to be similar to other analytical methods leveraging the specificity and sensitivity of the isotope-labeled internal standards. The value of the proposed method is especially revealing in its ability to semi-quantify the presence of unknown fluorinated contaminants in water. The authors explained the sampling, sample preparation, instrumental analysis procedure, and the data treatment and screening credentials. It is to this reviewer the significant advance of the proposed method to perform the identification and semi-quantification of the trace amount of polyfluorinated organic contaminants in the absence of commercial database packages. Experimentally, readers should be able to follow the depicted procedure step-by-step to obtain an analytical output. The challenge, however, may rest upon the experience needed to use the ion chromatograph and ions fragmentation data to effectively and correctly screen the possible compounds. In general, the paper is well written, and all procedures are clearly depicted.

Major Concerns:

Again, the primary challenge for an experimentalist to follow the proposed method is the lack of experience needed to use the ion chromatograph and ions fragmentation data to effectively and correctly screen the possible compounds. This paper uses several conceptual diagrams to explain the steps and credentials for a valid data interpretation. Providing an example, step by step, with a real sample and analysis would significantly enhance the success rate of the knowledge dissemination.

Unfortunately, the validation of novel compound discovery is currently outside the range of novice use of mass spectrometry. An example data file and results are included in the supplemental information, and references are made to manuscripts detailing the confirmation of structures using mass spectrometry, which is beyond the scope of this manuscript.

Minor Concerns:

Text description of Figure 6 is missing.

In-text reference to Figure 6 was in the methods description. Figures have been renumbered in-text.

Reviewer #2:

Manuscript Summary:

General comments.

In the paper Identification of per-/polyfluorinated chemical species with a combined targeted and non-targeted screening high-resolution mass spectrometry workflow, authors describe a method able to detect fluorinated and others untargeted compounds by coupled solid phase extraction with hight resolution mass spectrometry HPLC.

Report is good discussed, unfortunately, tables are mistake, and some errors were reported.

Major Concerns:

Authors report only Internal Standards. Why authors not use standard in order to monitor recovery?

PFAS standards were used to construct spike blanks for recovery assessment in step 1.1.2. Recovery assessment does not affect quantitation for IS spiked, extracted calibration curves and is relegated quantitative method development, where it can affect limit of detection and method reporting limits.

Where are Tables?

Please add a paragraph concerning to material and methods. Moreover, please add the instrument used.

JoVE materials table is included, which indicates instrumental platforms and a summary of reagent materials

Line 5 line 81

Please, add some recent references concerning to Online Solid-Phase Extraction and perfluorinated compounds

Page 2 line 124.

Have authors verified absence of fluorinated substances from Laboratory deionized? Often fluorinated compounds were detected in DI water.

Our laboratory water has been validated as being PFAS free, text to this effect has been added to the manuscript.

Page 3 line 144.

Are authors sure that nitric acid does not cause the oxidation of some untargeted compounds?

Nitric acid oxidation of traditional organic compounds is possible, PFAS materials are typically non-reactive under mild acidification.

Page 3 line 163

Please, report low levels used in calibration curve different from 0.

Calibration levels are to be determined by the experimentalist on the basis of desired quantitation range and the sensitivity of their instrumental platform.

Page 3 line 171

Add stable isotope labeled PFAS standard to approximate the midpoint of the LC-MS standard curve (e.g. 100 ng/L).

In line 163 authors reported that calibration curve ranged from 0 to 100 ng/L.

In line 163 the example calibration range was from zero to **1000 ng/L**. 100 ng/L undershoots the midpoint of that calibration curve but was a hypothetical example.

Page 4 line 177.

For analyses concerning to monitoring activities, samples must not be filtered. Moreover, authors not reported filter diameter. On another hand, by using SPE techniques, filtration can be avoided.

Pre-filtration is used to prevent clogging of the SPE cartridge system for dirty surface water samples, matched extracted calibration curves eliminate filtration related biases in quantitation. The filter diameter is not an analytically relevant parameter as it only affects the backpressure of filtration. However, the filter used is listed in the JoVE Materials table.

Minor Concerns:

have authors used column trap to avoid PFAS contamination from system?

Modifications to the LC system, including the PFAS hold-up column, are mentioned in the JoVE Materials table.

Reviewer #3:

Manuscript Summary:

The manuscript "Identification of per-/polyfluorinated chemical species with a combined targeted and non-targeted screening high-resolution mass spectrometry workflow" presents a protocol, as the title indicates, for targeted and non-targeted identification of per-/polyfluorinated compounds (PFASs) using high-resolution mass spectrometry. The article responds to the need of having forward-looking methodologies that can detect emerging and unexpected compounds (in this case PFASs and related compounds), monitor these species over time, and resolve details of their chemical structure.

Minor Concerns:

The manuscript is clear, well organized and in general very informative. Even if objectives are not clearly presented, they can be easily deduced from text. The references used are updated and they support properly the introduction and discussion sections. The number of figures and tables is correct. My only comment is related to these figures. They are not presented in ascending order (first figures 6 and 7 are cited and later figures 1, 2 , 3…). Additional information about them would also be of interest. For instance in figure 1, which are the perfluorinated ether standards used for obtaining this total ion chromatogram (TIC), or a small description of the fluorochemical production site which upstream and downstream surface waters were collected and extracted to obtain the overlaid TIC presented in this figure. This is a very good paper and I only suggest MINOR changes before it can be accepted for publication in the Journal of Visualized Experiments.

Figure references have been updated as appropriate. The Figure 1 TIC is a wide-scan total ion chromatogram and is not constructed from standards. Further description of the fluorochemical production site is not included due to privacy concerns, but references are provided in

Reviewer #4:

Manuscript Summary:

This work describes a method to extract, identify, and potentially quantify PFAS found in environmental water samples. The authors describe a method that allows for the detection of PFASs not included in the standard EPA 537 method. This work is an excellent candidate for visual representation given the current climate surrounding PFAS in the United States. These methods will help researchers to implement these methods when typical journal articles do not provide detailed information.

Major Concerns:

None

Minor Concerns:

- Providing a couple of lists or example lists of a full set of method blanks, calibration curve, etc might be useful for researchers new to LC-MS/MS.

An example worklist has been added as a table.

- Add to the discussion of the types of PFAS extracted in this method - talk about specifics of the types of compounds that are more likely to be extracted

A brief discussion of these points occurs in the introduction (Lines 83-91 and 110-113)

- Highlight potential places in the method where PFAS contamination is particularly likely

A few key points of contamination are now included as notes