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Matrix Matching or Isotope Dilution? A Comparison of Two Quantitation Approaches to Determine PFAS in Dairy Milk

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This is an Application Brief and does not contain a detailed Experimental section.

Abstract

Application of water and biosolids contaminated with per- and polyfluorinated alkyl substances (PFAS) to produce feed crops for livestock has greatly impacted the quality of dairy products farmed from these animals. Thus, it is crucial to have sensitive and accurate analytical methods to monitor PFAS levels in dairy milk. The QuEChERS method was implemented for extraction of PFAS using DisQuE products followed by highly sensitive LC-MS/MS analysis on ACQUITY™ I Class PLUS coupled to Xevo™ TQ-XS. The performance of the method was evaluated for locally purchased dairy milk and proved to be accurate and robust for a range of thirty PFAS compounds of varying chemistry classes. Additionally, matrix matched calibration and solvent-based isotope dilution calibration curves were compared, with both proving to be appropriate for calculating compound concentrations in samples with no significant difference observed between the two methods.

Benefits

- A time efficient and simple extraction of thirty PFAS from dairy milk utilizing a QuEChERS extraction method and dSPE cleanup

- Sensitive analysis on the Xevo TQ-XS to detect PFAS at ng/L levels to effectively monitor PFAS exposure in dairy milk to match the levels detected in samples worldwide
- Increased confidence in results with the utilization of the PFAS Kit for liquid chromatography (LC) modification to isolate possible system and solvent contaminants
- Flexibility in quantitative approach (matrix matched or isotope dilution) can be decided based upon what works best for a laboratory when considering number of different matrix types being run as well as budget and availability of internal standards

Introduction

Industrial and commercial usage of PFAS has impacted more than just the water we consume. Dietary intake has been recognized as an important PFAS exposure pathway for the general population.[1] Contaminated water is also used to grow feed crops for and hydrate livestock animals. Additionally, use of biosolids (organic matter removed during sewage treatment) as fertilizers on livestock feed crops has become a major source of PFAS contamination in not only the meat produced from these animals, but the dairy products as well. A recent notable contamination event has impacted Maine dairy farmers in the United States requiring them to dispose of all their milk products, slaughter remaining livestock, and causing complete loss of their livelihood.[2] Due to the lack of regulation of PFAS in biosolids and food, this contamination event has led the state of Maine to create their own screening levels for select PFAS in water, soil, milk, beef, and feed crops.[3] For reference, the Maine Action Level in milk is currently for PFOS only and is set at 210 ng/L (ppt). PFAS contamination of milk is a global issue with a quick literature search returning publications on PFAS detection in milk in Poland (5 PFAS detected in range of 20–980 ng/L), South Africa (fifteen PFAS detected in range of 10–2,100 ng/L), and Italy (up to 97 ng/L PFOS detected), to highlight just a few.[4,5,6]

With so many known instances of dairy being contaminated by PFAS around the world, it is necessary to have analytical techniques available to monitor milk and dairy products that are sufficiently sensitive and accurate. A simple extraction technique that can be employed for PFAS extraction in milk is QuEChERS (Quick, Easy, Cheap, Effective, Rugged, Safe). This technique is widely used for the extraction of pesticides from food but is often adopted for determination of other contaminants. QuEChERS uses salts and acetonitrile to extract compounds of interest through a salting out and phase separation mechanism coupled with a dispersive solid-phase extraction (dSPE) cleanup step. This fast and simple extraction technique was evaluated for extraction of PFAS from dairy milk, with analysis using ACQUITY I Class PLUS coupled to Xevo TQ-XS. Two calibration and quantitation methods (matrix matching and isotope dilution using solvent calibration) were compared using these samples as an example.

Experimental

Method Information

The same QuEChERS, dSPE protocol, and instrument methods used for edible produce (described in Waters Application Note 720007333)[7] were used for the analysis of PFAS in milk. Milk acquired locally was spiked with thirty PFAS at 0.1 ng/g and 1.0 ng/g (0.025 and 0.25 ng/mL in vial, respectively) to evaluate the performance of the method.

Prior to extraction a mix of twenty stable isotope labelled internal standards were spiked into the sample to use as recovery adjustment for the isotope dilution calibration approach. Additionally, a mix of three isotope labelled internal standards was spiked into all samples after extraction to use as injection standards. For the matrix matched calibration approach, only two of the extraction standards ($^{13}C_8$-PFOA and $^{13}C_8$-PFOS) were used during quantitative calculations.

Results and Discussion

A chromatogram showing the transitions used for quantitation of each PFAS spiked into milk at the 0.1 ng/g level can be seen in Figure 1. It is worth noting that the typical quantitative (562.9>269) and qualitative (562.9>518.9) MRMs for perfluoroundecanoic acid (PFUnDA, depicted as Peak 8) were switched for this matrix due to a closely eluting isobaric peak from the matrix in the typically used quantitative MRM.

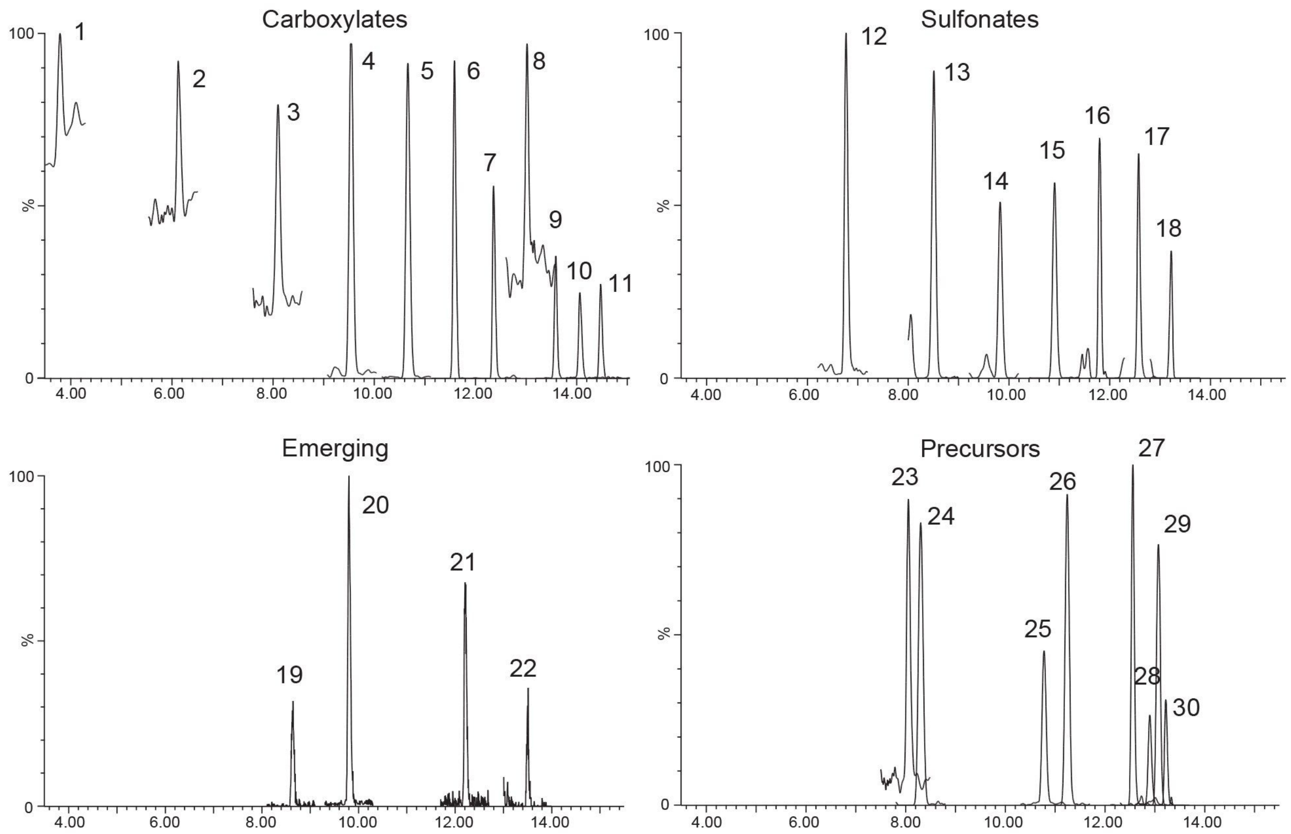
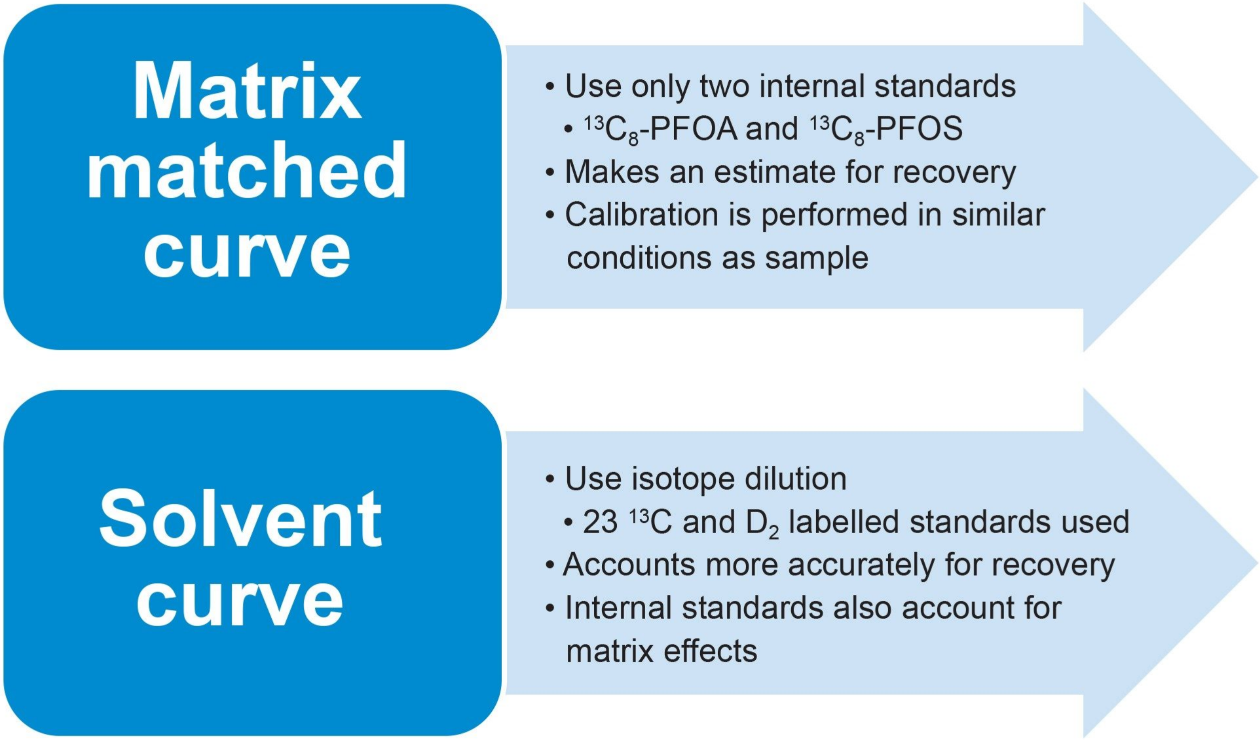


Figure 1. Extracted ion chromatograms of the quantitation ion for each PFAS in the 0.1 ng/g spike in milk. Peak identifications are as follows: (1)PFBA (2)PFPeA (3)PFHxA (4)PFHpA (5)PFOA (6)PFNA (7)PFDA (8)PFUnDA (9)PFDoDA (10)PFTriDA (11)PFTreDA (12)PFBS (13)PFPeS (14)PFHxS (15)PFHpS (16)PFOS (17)PFNS (18)PFDS (19)HFPO-DA (20)ADONA (21)9Cl-PF3ONS (22)11Cl-PF3OUdS (23)4:2 FTS (24)FBSA (25)6:2 FTS (26)FHxSA (27)8:2 FTS (28)NMeFOSAA (29)NEtFOSAA (30)FOSA.

Matrix effects can be very challenging during analysis of foodstuffs using liquid chromatography with tandem mass spectrometry (LC-MS/MS), as they can have a significant impact on quantitation. During this evaluation, two types of calibration curve options designed to mitigate the impact of matrix effects were assessed: solvent calibration using isotope dilution and matrix matched calibration. While matrix matching can be preferred in food analysis to provide calibration conditions closely matched to the sample type, this can prove impractical when working with many different matrix types in a single batch. To compensate for any potential matrix induced response differences when using solvent curves, an extensive mix of stable isotope labelled internal standards were used for this approach. For isotope dilution quantitation, a mix of 23 internal standards were spiked before sample extraction (extraction standard) and after sample extraction (injection standard). Matrix-matched standards were prepared in blank milk extract using only two internal standards spiked into samples prior to extraction. Figure 2 summarizes the difference between the approaches as well as internal standards used for each.



Matrix matched curve

- Use only two internal standards
  - $^{13}C_8$-PFOA and $^{13}C_8$-PFOS
- Makes an estimate for recovery
- Calibration is performed in similar conditions as sample

Solvent curve

- Use isotope dilution
  - 23 ^{13}C and D_2 labelled standards used
- Accounts more accurately for recovery
- Internal standards also account for matrix effects

Figure 2. Summary of the two different types of calibration curves evaluated during this analysis.

Overall, similar results in extracted milk samples were observed using each calibration approach. A comparison of the peak area response between the solvent and matrix curves across four different PFAS chemistry groups can be seen in Figure 3, demonstrating that the response overall was not impacted by the presence of the milk matrix. In regard to recovery, the observed range calculated using the solvent curve (relative recovery based on internal standard correction) was a little wider (58–134% for 0.1 ng/g spike and 58–109% for 1.0 ng/g spike) than the absolute recovery of the matrix matched curve (76–130% for 0.1 ng/g spike and 68–96% for 1.0 ng/g spike). The overall trend was still the same with recovery decreasing as chain length increased, as can be seen in Figure

4. Besides the minimum and maximum recoveries in the range, no significant difference was observed using each method of calibration. The recovery values also demonstrate that the QuEChERS method of sample preparation is suitable for extraction of PFAS from milk based on analytical acceptance guidance from the US FDA (40–120%) and the EURL (65–135%).[8,9]

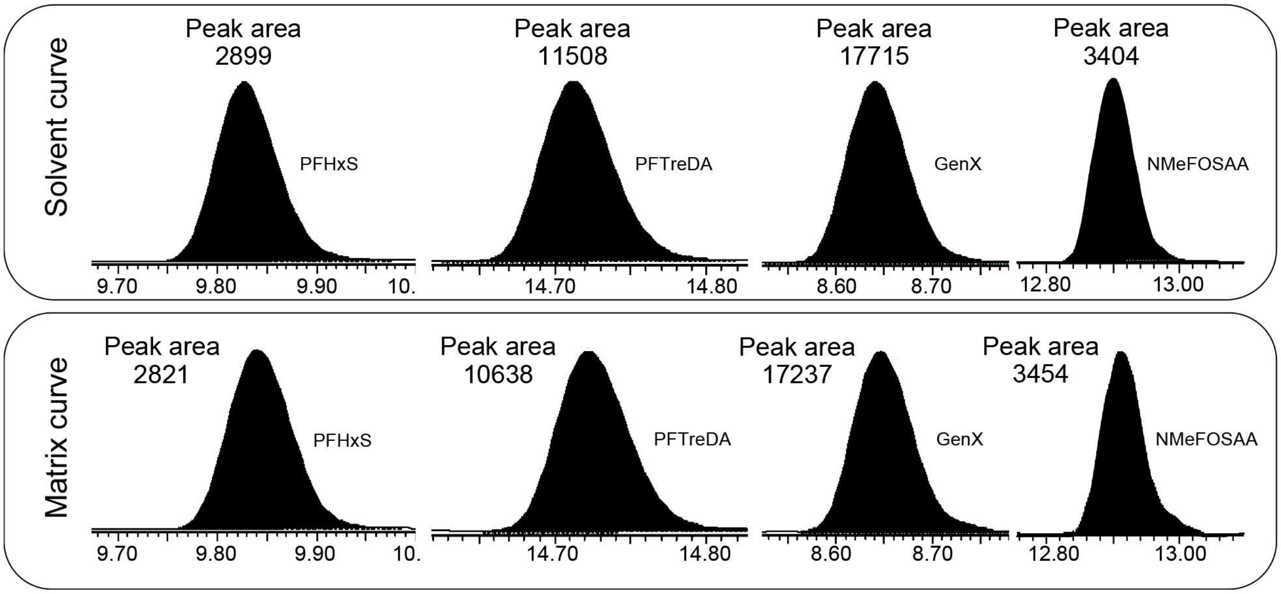
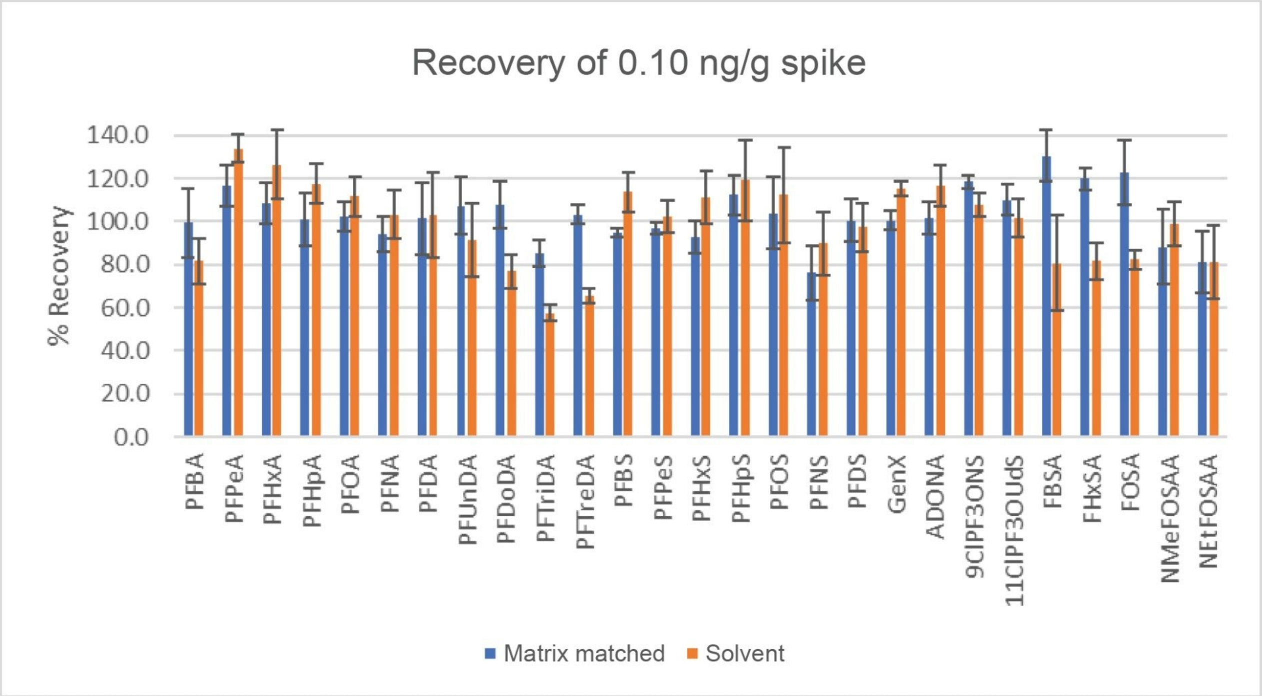


Figure 3. Peak area comparison of PFHxS, PFTreDA, GenX, and NMeFOSAAin 100 ng/L points of both the solvent and matrix matched curves.



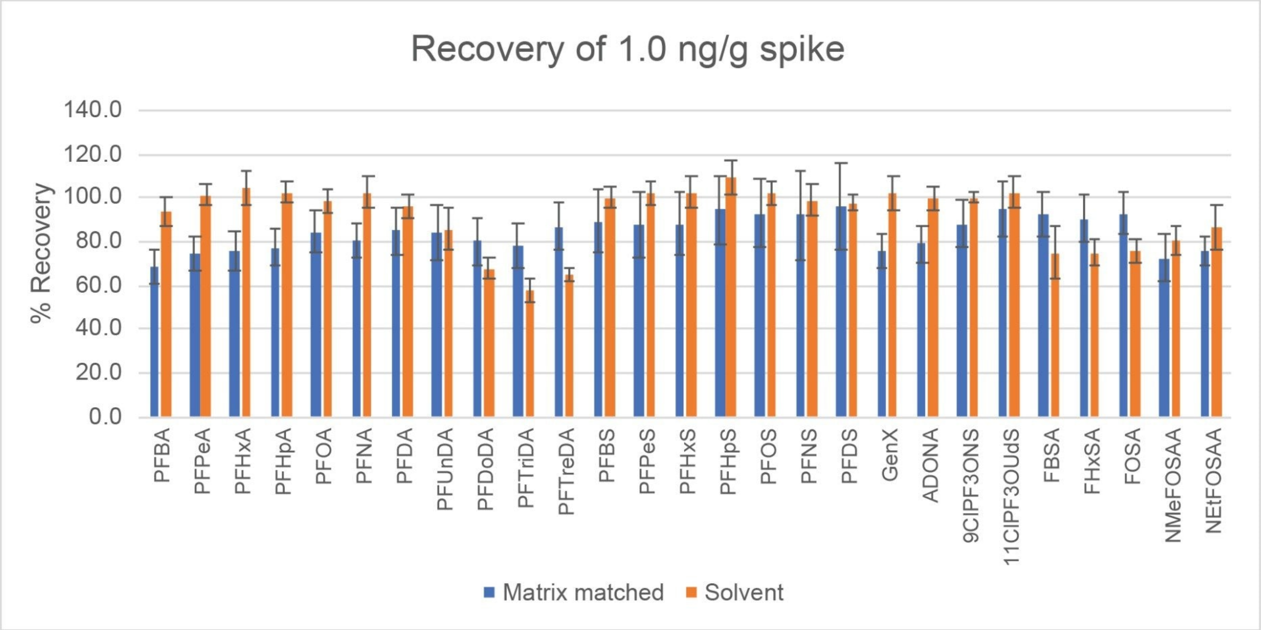


Figure 4. Percent recovery of each PFAS spiked in milk at (top) 0.10 ng/g and (bottom) 1.0 ng/g.

Finally, the accuracy of each calibration method was assessed by comparing the calculated concentrations of known concentrations spiked into milk samples (n=5), with results shown in Figure 5. Accuracy was good for both calibration methods with mean percent accuracy of 85% and 97%, for matrix matched and solvent curve respectively. The solvent standard curve using isotope dilution did have a slightly larger spread of accuracy, but an overall higher mean accuracy. The low-end outliers in the case of the solvent curve were from the compounds that didn't have an exact isotope labelled counterpart for correction. The lower mean percent accuracy for the matrix matched curve was expected since percent recovery is not accounted for during concentration calculation. The repeatability (%RSD) of the calculated concentrations was also slightly better for the solvent curve using isotope dilution, with %RSD under 15%. For the matrix matched curve, the %RSD of calculated concentrations were almost all <20%, except for PFNS. Again, this was expected as the solvent curve using isotope dilution calculation corrects more for any variation derived from sample specific recovery and matrix effects. In either case, the accuracy of both options is acceptable based on guidance from the US FDA (RSD ≤22%) and EURL (RSD ≤25%).[8,9]

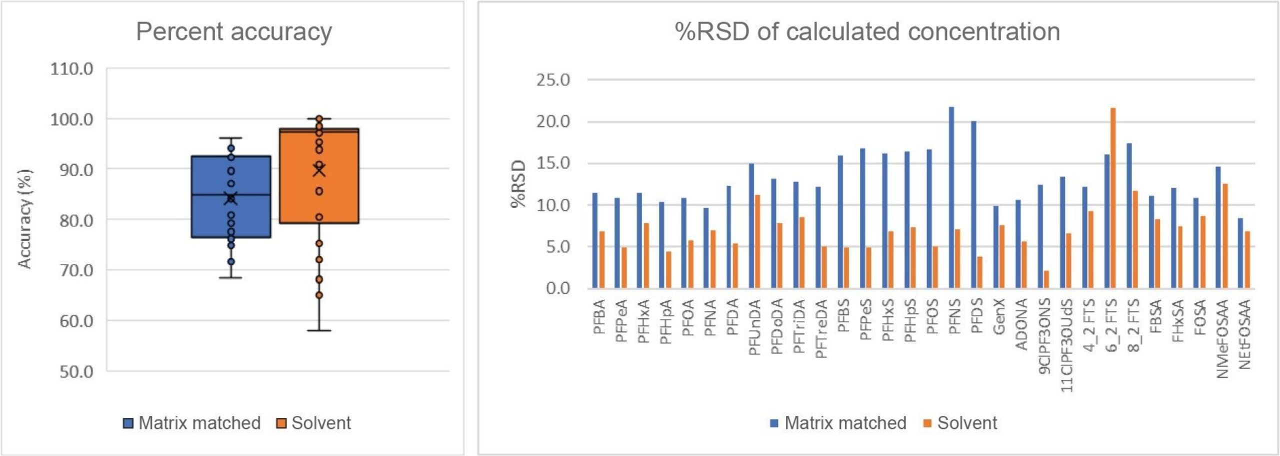


Figure 5. Percent accuracy and %RSD of calculated concentrations for all compounds using both the matrix matched and solvent curve calibration approaches.

Conclusion

With increasing awareness of the impact of PFAS in food, it is important to have methods that can detect these compounds in a variety of food types. PFAS analysis in dairy milk was successfully performed by applying the same QuEChERS based extraction approach previously used for the analysis of edible produce. Using QuEChERS extraction is fast and easy, utilizing small sample amounts and small volumes of organic solvents. When acquiring the LC-MS/MS data, two common approaches to calibration were explored and results compared. Matrix matching using a reduced number of internal standards was compared with solvent-based calibration curves containing as many direct analog internal standards as available. Calculated recovery values were similar overall using both methods, with measured relative recoveries typically >60% for the solvent standard approach, and absolute recovery >70% for the matrix matching approach. The long chain PFAS experienced lower recoveries, with all other compounds having a recovery of ≥80% using both methods. Accuracy of calculated concentrations for known spiked concentrations of PFAS were also compared for each calibration method. The mean percent accuracy for matrix matching was 85%, whereas the solvent calibration using isotope dilution was 97%. The higher accuracy was expected since the isotope dilution calculation takes into account recovery when reporting calculated concentrations. Finally, the repeatability of the measurements was found to be slightly better for isotope dilution (<15% except for one outlier) than for matrix matching (<20%). Overall, these results demonstrate that using either calibration approach, the QuEChERS method followed by LC-MS/MS analysis using ACQUITY I Class and Xevo TQ-XS allows for high confidence in results for a rapid and easy analysis of PFAS in milk. Calibration approach can be dependent upon what works best for a laboratory when considering how many different matrix types are run as well as budget, availability of internal standards, and access to PFAS free matrix blanks. Regardless of which approach is used, this method can be successfully implemented to confidently ensure the safety of milk products in locations that may have been impacted by PFAS contamination.

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