

Note d'application

## QuEChERS Extraction of Per- and Polyfluoroalkyl Substances (PFAS) from Edible Produce with Sensitive Analysis on Xevo TQ-XS

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### Abstract

The same sources of environmental per- and polyfluorinated alkyl substances (PFAS) exposure can also lead to contamination in food sources. Cultivating produce using PFAS contaminated water and soils can lead to the uptake of these compounds into the edible fruits and vegetables portions of plants. Thus, it is beneficial to have a straightforward method to monitor the occurrence of PFAS in produce. For this work, the FDA C-010.01 method based on the QuEChERS extraction method was implemented for extraction of PFAS using DisQuE dispersive solid phase extraction (dSPE) products followed by highly sensitive LC-MS/MS analysis on ACQUITY UPLC I-Class PLUS coupled to Xevo TQ-XS. The method was evaluated in five different commodity types including lettuce, strawberry, cranberry, carrot, and potato. With a few minor adjustments to the FDA method, this approach to PFAS analysis in produce proved to be accurate and robust for a range of 30 PFAS compounds of varying chemistry classes.

### Benefits

- A time efficient and simple extraction of PFAS from edible produce utilizing a QuEChERS extraction method
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and dSPE cleanup

- Sensitive analysis on the Xevo TQ-XS to detect PFAS at sub-ng/g levels to match detected concentrations published in reports by EFSA and FDA
- Confidence in results with the utilization of the PFAS Kit for LC modification to isolate possible system and solvent contaminants

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## Introduction

The environmental impact of per- and polyfluoroalkyl substances (PFAS) is readily known from the prevalent usage of these compounds in everyday products. Often, environmental issues also impact our food sources.

In the case of agricultural produce, PFAS impacted water and soil used for irrigation and growing crops can result in contamination. Studies show that edible plants do uptake PFAS, with higher uptake of short chain PFAS (PFBA and PFPeA) in the edible portions and a wider range of PFAS uptake in the roots and stalks/stems of the plants.<sup>1,2</sup> Since irrigation water is most typically also drinking water or ground water, contamination can be introduced through any of the environmental contamination pathways (manufacturing discharge, firefighting foam, landfill leachate, etc).<sup>3</sup> Soil contamination can occur from similar mechanisms, but the use of biosolids as fertilizers has become a major concern for crop contamination.<sup>4</sup>

Although some countries impose regulatory or advisory limits on the concentration of PFAS in water, limits for PFAS have yet to be set in biosolids or food. The European Food Safety Authority (EFSA) have evaluated and published data on human health risks due to presence of PFAS in food. The most recent report published in 2020 concluded that, of the 27 PFAS evaluated, fish, fruit, and eggs contributed the highest levels of exposure.<sup>5</sup> Data submitted for this study utilized a range of extraction and analysis techniques. The US Food and Drug Administration (FDA) monitor contaminants in highly consumed foods in their Total Diet Study.<sup>6</sup> To be able to include PFAS in this study, they created and validated an extraction method for PFAS in foods.<sup>7</sup> This method (FDA C-010.01)<sup>8</sup> utilizes a QuEChERS extraction, followed by dispersive solid phase extraction (dSPE) clean up. QuEChERS (Quick, Easy, Cheap, Effective, Rugged, Safe) is a widely used extraction technique first created for extraction of pesticides from food and is often adopted for determination of other contaminants. This technique uses salts and acetonitrile to extract compounds of interest through a salting out and phase separation mechanism. This fast and simple extraction technique was evaluated for extraction of PFAS from a variety of

produce samples, with analysis using ACQUITY UPLC I-Class PLUS coupled to Xevo TQ-XS.

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## Experimental

### Sample Preparation

Produce samples were purchased at a local grocery store. Strawberries, cranberries, romaine lettuce, whole carrots, and russet potatoes were used in this study. The edible portions of each produce item were homogenized using a Ninja kitchen blender. Samples were stored in a freezer (-20 °C) and thawed in a refrigerator (4 °C) overnight prior to extraction.

5 grams of each sample were extracted using DisQuE AOAC QuEChERS salts. A suite of 20 isotope labeled standards purchased from Wellington Laboratories (MPFAC-24ES + M3HFPO-DA) were spiked into each 5-gram sample as surrogates prior to extraction at a concentration of 1 ng/g. The full QuEChERS extraction and dSPE cleanup is outlined in Figure 1. MPFAC-C-IS was spiked into each sample prior to injection as an internal standard at a final concentration equal to 1 ng/g ( 0.25 ng/mL in vial). Shaking steps were performed using a SPEX Sample Prep Geno/Grinder. The surrogate and internal standards were used in an isotope dilution approach for calculating native PFAS concentrations. The surrogate standards correct the calculation for recovery since they are present in the sample through all sample preparation. The internal standards, only present after extraction, correct the surrogates for any variation during injection.

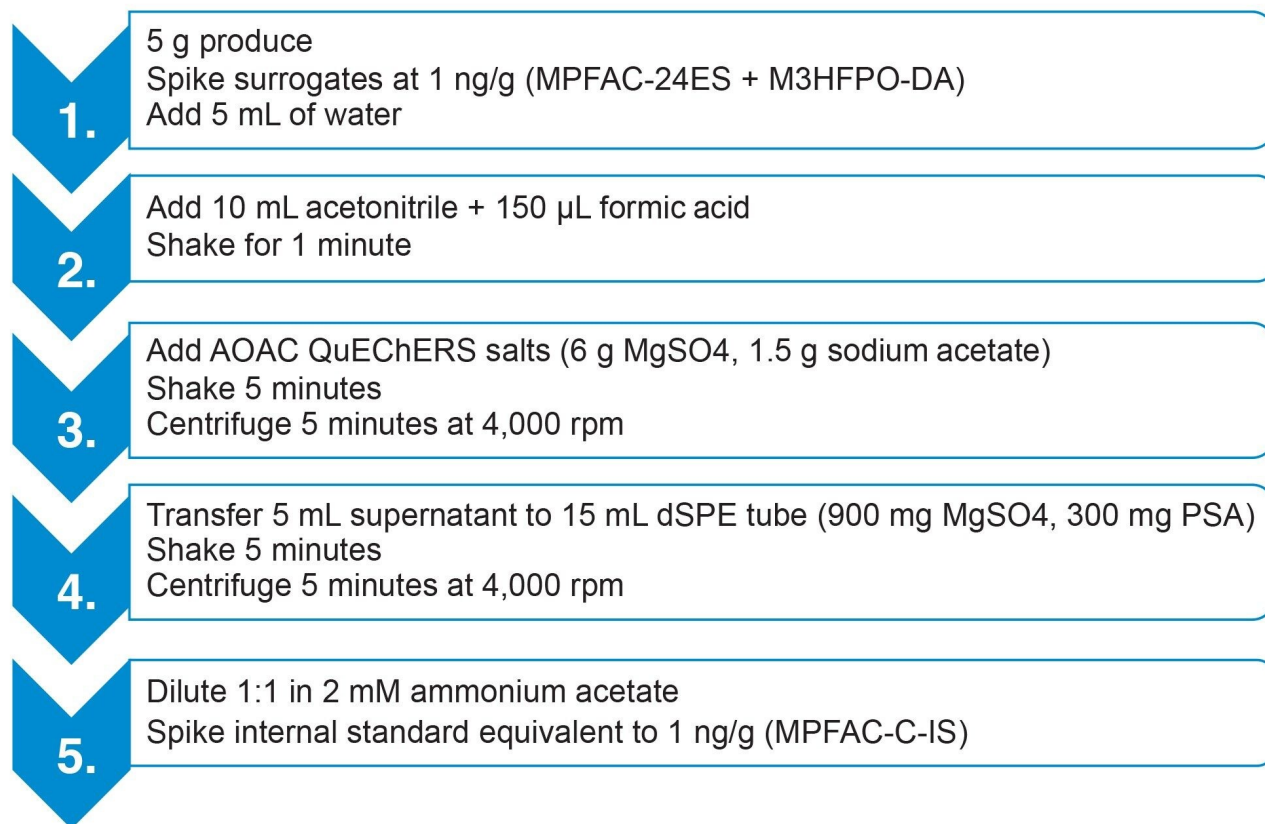


Figure 1. Full QuEChERS method for extraction of PFAS from produce samples using DisQuE AOAC salts (p/n: 186006812) and 15 mL dSPE tubes (p/n: 186008077).

A solvent calibration curve in the range of 0.01 ng/mL–5 ng/mL (equivalent to 0.04–40 ng/g) was used. With the presence of the surrogates and internal standards, matrix matching is not necessary, but is an option in place of performing the isotope dilution method.

## Method Conditions

### LC Conditions

LC system: ACQUITY UPLC I-Class PLUS FTN  
with PFAS Analysis Kit

Vials:	Polypropylene autosampler vial with polyethylene cap
Column(s):	ACQUITY UPLC BEH C <sub>18</sub> 2.1 x 100 mm, 1.7 μm
Column temp.:	35 °C
Sample temp.:	4 °C
Injection volume:	10 μL
Flow rate:	0.3 mL/min
Mobile phase A:	Water + 2 mM Ammonium acetate
Mobile phase B:	Methanol + 2 mM Ammonium acetate

## Gradient Table

Time (min)	%A	%B	Curve
0	95	5	initial
1	75	25	6
6	50	50	6
13	15	85	6
14	5	95	6
17	5	95	6
18	95	5	6
22	95	5	6

## MS Conditions

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MS system:	Xevo TQ-XS
Ionization mode:	ESI-
Capillary voltage:	0.50 kV
Desolvation temperature:	350 °C
Desolvation flow:	900 L/hr
Cone flow:	150 L/hr
MS method:	See Appendix for MS Method information

## Data Management

Chromatography software:	MassLynx v4.2
MS software:	MassLynx v4.2
Informatics:	MassLynx v4.2 with TargetLynx 4.2

## Adjustments made to the FDA Guidance Method

The FDA method uses the unbuffered version of QuEChERS. The unbuffered salts (MgSO<sub>4</sub> and NaCl) were compared to two different buffered methods (AOAC and CEN)<sup>9,10</sup> to determine the most effective extraction conditions for PFAS using cranberry as the matrix. The bar graphs in Figure 2 demonstrate examples of recovery using the three salt combinations. There were some variations in recovery for certain compounds, but in general the three salt combinations extracted PFAS equally and the AOAC method was selected for further evaluation.

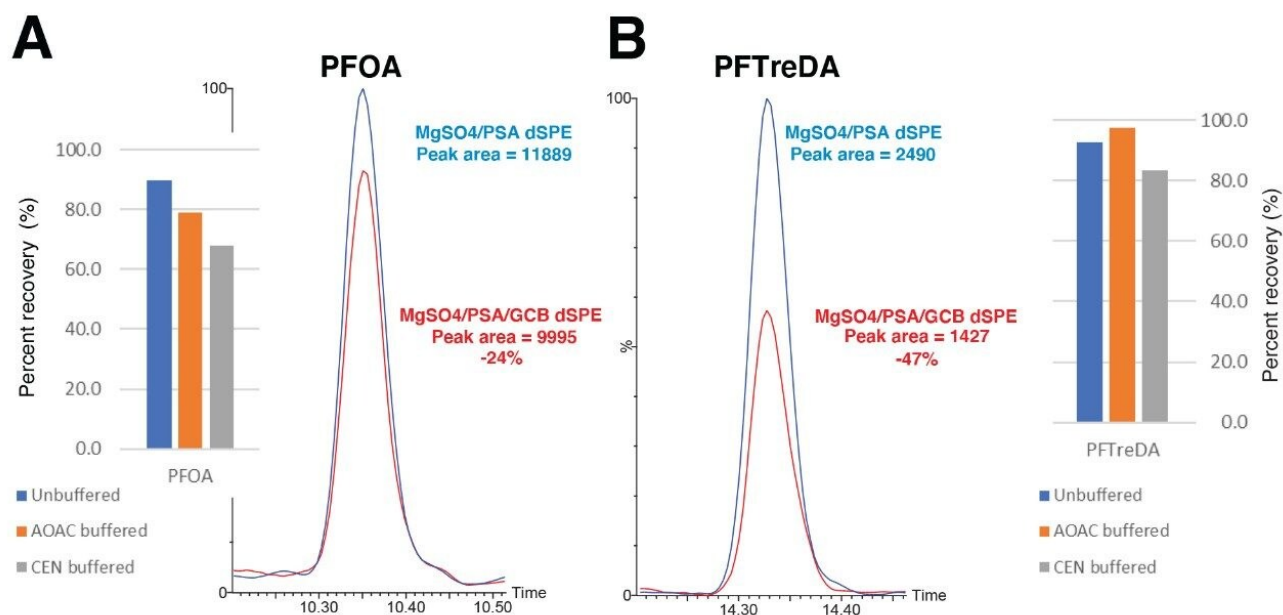


Figure 2. Evaluation of different QuEChERS salts represented as recovery in bar graphs and the effects of including GCB in the dSPE cleanup shown in peak overlays. (A) results for PFOA and (B) PFTreDA.

The sorbents used for dSPE were evaluated, with focus on the amount of graphitized carbon black (GCB) added. For this comparison the rest of the dSPE sorbents remained consistent, using 900 MgSO<sub>4</sub> and 300 PSA (per 15 mL tube). This configuration was tested with and without 150 mg GCB since GCB is known for strong adsorption of compounds which can lead to reduced recovery of some compounds. The overlaid peaks in Figure 2 show the effect of peak response with and without GCB. Peak response (and therefore recovery) decreased with the use of GCB. This effect increased as C-F chain length increased. Therefore, GCB was eliminated from the final dSPE clean up method.

The final adjustment that was made to the published FDA method was to add a dilution step prior to LC-MS/MS analysis. This was to improve the peak shapes of the early eluting PFAS since injection in 100% acetonitrile causes peak splitting and widening for compounds like PFBA, PFPeA, and 4:2 FTS. Figure 3 shows the benefit of adding the dilution step for the early eluters. This figure also demonstrates that even though there is some reduction in response for the later eluters (ex. PFNA), it doesn't negatively impact the sensitivity significantly.

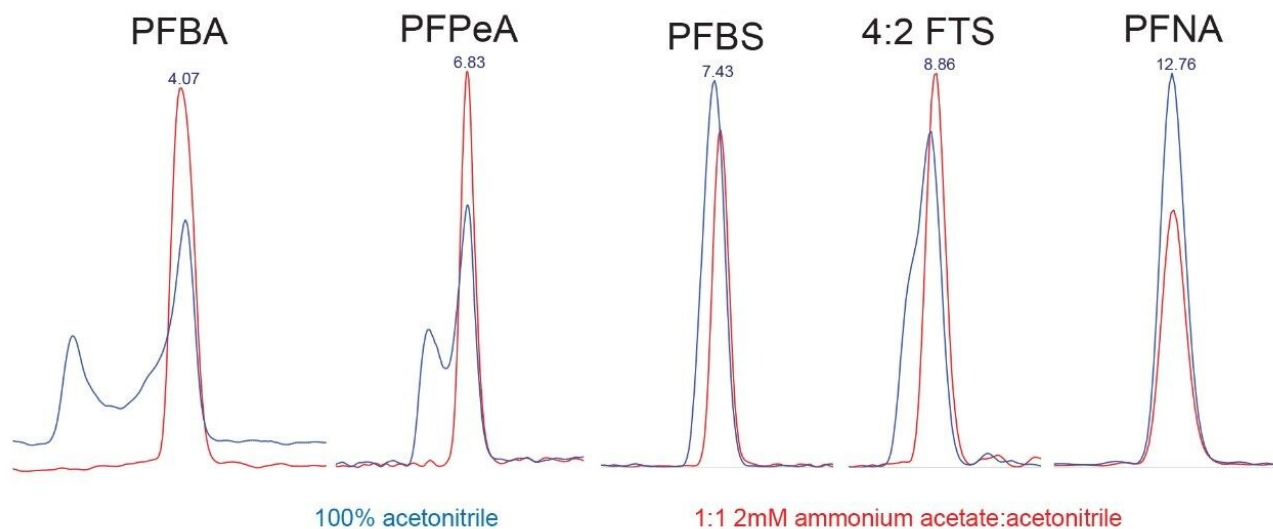


Figure 3. Demonstration of the peak shape correction gained from sample dilution. Blue peaks are undiluted samples and red peaks are diluted 1:1 with 2 mM aqueous ammonium acetate.

## Results and Discussion

The FDA guidance method was only validated using lettuce as the only representative produce sample. This study extended the scope to five different commodity classes, as described in Table 1. The number of analytes was increased from the original 16 in the FDA method to a total of 30 PFAS including the following compounds; Carboxylates: C<sup>4</sup>-C<sup>14</sup>; Sulfonates: C<sup>4</sup>-C<sup>10</sup>; Emerging: GenX, ADONA, 9Cl-PF3ONS, 11Cl-PF3OUdS; Precursors: FBSA, FHxSA, FOSA, NMeFOSAA, NEtFOSAA, 4:2 FTS, 6:2 FTS, 8:2 FTS.



Produce	Commodity class	Spike level	Carboxylates	Sulfonates	Emerging	Precursors
Romaine lettuce	High water	0.1 ng/g	95-118 (9.4)	78-118 (17)	108-121 (12)	87-135 (5.7)
		1.0 ng/g	80-90 (4.2)	92-101 (4.4)	86-95 (5.4)	69-99 (3.0)
		5 ng/g	84-95 (4.9)	99-104 (5.4)	94-103 (6.1)	82-108 (6.2)
Russet potato	High starch	0.1 ng/g	72-121 (7.0)	95-111 (12)	95-104 (10)	81-110 (10)
		1.0 ng/g	77-93 (6.1)	92-102 (5.0)	85-96 (6.5)	68-103 (3.8)
		5 ng/g	71-84 (4.0)	76-83 (3.5)	74-85 (4.6)	62-85 (4.1)
Whole carrot	Low water	0.1 ng/g	78-132 (6.6)	94-110 (12)	90-101 (9.5)	94-108 (8.9)
		1.0 ng/g	79-98 (6.9)	91-103 (5.7)	88-106 (6.2)	79-102 (5.0)
		5 ng/g	75-89 (8.0)	80-87 (4.0)	77-86 (5.6)	70-89 (4.5)
Strawberry	High sugar	0.1 ng/g	89-110 (10)	89-118 (7.9)	100-111 (14)	104-128 (6.3)
		1.0 ng/g	82-98 (3.2)	92-98 (4.2)	86-101 (5.6)	75-104 (3.0)
		5 ng/g	90-99 (7.4)	99-105 (6.5)	95-105 (7.3)	86-105 (7.3)
Cranberry	High acid and sugar	0.1 ng/g	85-99 (8.6)	86-99 (11)	87-102 (19)	83-106 (8.1)
		1.0 ng/g	85-101 (8.3)	95-103 (5.6)	91-101 (8.5)	80-101 (4.8)
		5 ng/g	65-93 (5.4)	80-89 (2.7)	79-93 (5.1)	70-89 (3.3)

Table 1. Range of percent recoveries (%) in each commodity at three different spike concentrations for n=5 extractions at each concentration. Values denoted as (n) are the average %RSD.

Five replicates of each produce commodity were spiked at 3 concentration levels; 0.1 ng/g, 1.0 ng/g, and 5 ng/g. Example chromatograms of the extracted quan ion trace of each PFAS pre-spiked into potato at the lowest level of 0.1 ng/g can be seen in Figure 4. Additionally, branched and linear isomers for PFHxS, PFOS, NMeFOSAA, and NEtFOSAA were detectable in matrix, as demonstrated in Figure 5 for PFOS in matrix at 0.05 ng/g.

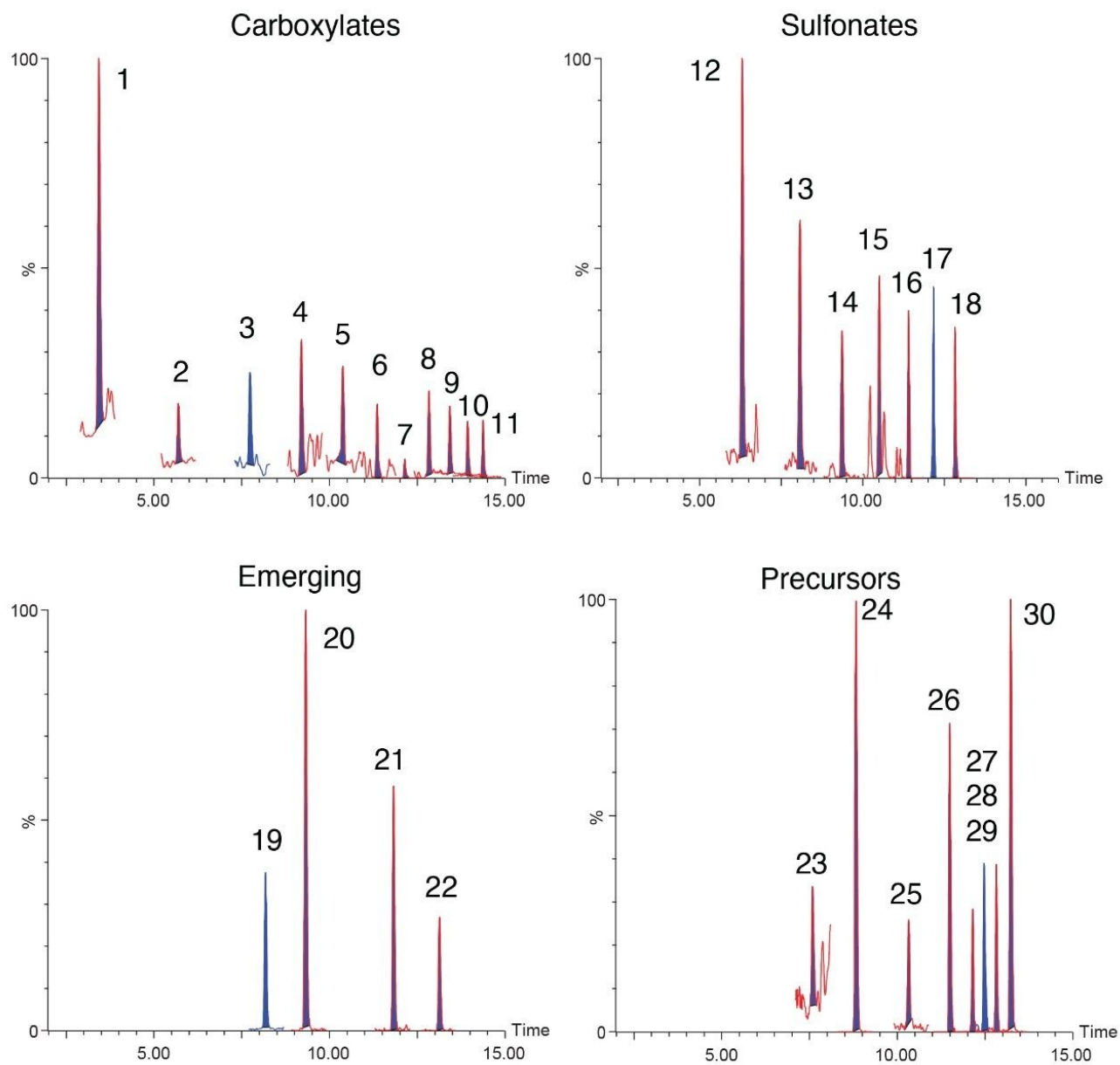


Figure 4. Extracted ion chromatograms of the quantitation ion for each PFAS in the 0.1 ng/g spike in potato. Peak identifications are listed in the Appendix.

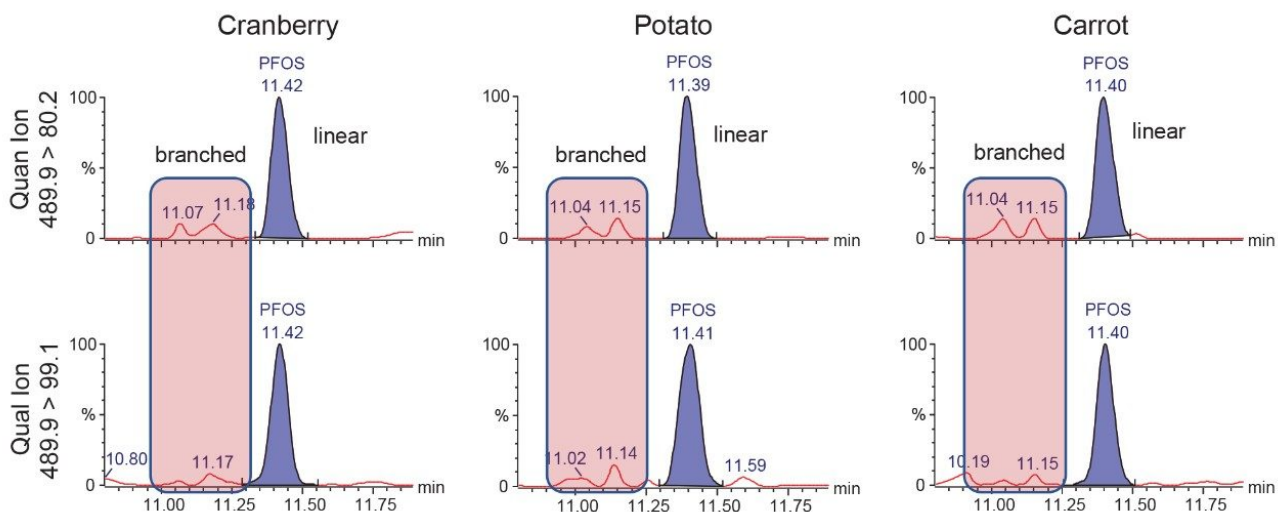


Figure 5. Detection of branched and linear PFOS isomers in 0.05 ng/g cranberry, potato, and carrot matrix.

Recovery was calculated by comparing response values of a pre-spike matrix sample to a matrix blank post spiked at the same concentration. The only correction that was performed prior to the recovery calculation was internal standard correction (internal standard spiked after sample preparation to correct for system variance and matrix effects). The ranges of measured recovery of all compounds at each concentration level are shown in Table 1 for each commodity, separated by PFAS class, with the average %RSD represented in parentheses. Overall, across all commodity groups, recoveries were in the range of 62–135%, with mean recoveries of 72–113%. The mean recoveries for each PFAS group at each concentration in all commodities are displayed in Figure 6. All commodities spiked at 1.0 and 5.0 ng/g had a %RSD below 10%. The %RSD at the 0.1 ng/g spike were all below 20%. FDA guidance states an acceptable recovery range of 40–120% for concentrations at 1 ng/g and a maximum %RSD of 22%.<sup>11</sup> The reported recoveries fall into this acceptable range, with only a few outliers above 120% at the low spiked concentration. All of the %RSDs fall within the acceptable range as well. These experimental values indicate an accurate and reproducible extraction and analysis.

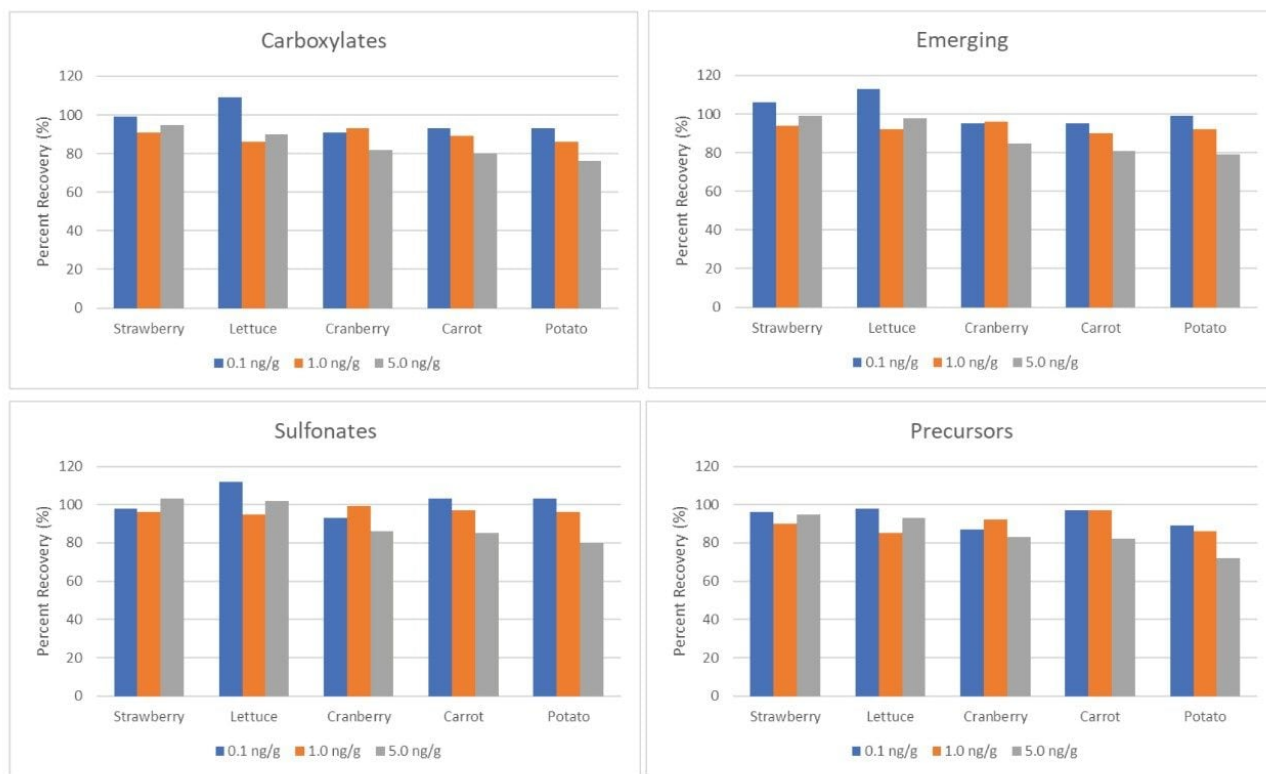


Figure 6. Mean percent recoveries for  $n=5$  extractions at each concentration level in each tested commodity.

To take into account any matrix enhancement or suppression that may impact the calculated concentration, as well as any losses during extraction, the isotopically labelled standards spiked into the samples prior to the QuEChERS extraction (surrogates) are used to perform isotope dilution calculations. The box and whisker plots in Figure 7 demonstrate how this correction calculates more accurate results, while using a solvent calibration curve, in the matrices with the greatest matrix effects; cranberry, carrot, and potato. The expected concentration of PFAS in these samples was 5 ng/g. Without using any isotope dilution correction, the mean concentrations in cranberry, carrot, and potato are 3.6, 4.0, and 4.2 ng/g, respectively. The spread of calculated concentrations is also wider with no correction. Using the surrogates for isotope dilution calculations, the mean concentrations are 4.7, 4.6, and 4.8 for cranberry, carrot, and potato, with a narrower range.

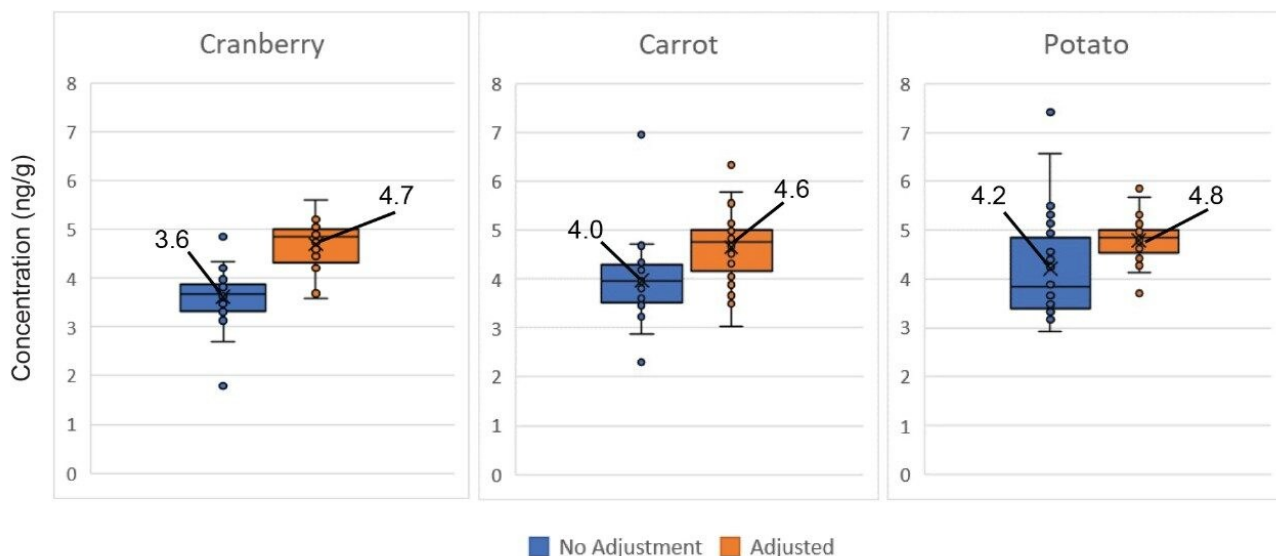


Figure 7. Using isotope labelled surrogates for isotope dilution calculations to perform concentration correction. Expected concentration is 5 ng/g. (blue) calculated concentrations without using surrogates to correct (orange) calculated concentrations using isotope dilution. Labeled data points are the mean.

## Conclusion

With a few minor adjustments to the extraction procedure, the QuEChERS-based FDA Method C-010.01 was successfully used to analyze PFAS in a variety of different edible produce samples. The QuEChERS extraction was fast and easy, utilizing small sample amounts and small volumes of organic solvents. The suite of PFAS evaluated was expanded to 30 compounds. Overall, the method performance was within the FDA guidance criterion when considering recovery, which translates to confidence in accuracy of results. The method was also determined to have good repeatability, having low %RSD values for replicate extractions. This method allows for high confidence in results for a rapid and easy analysis of PFAS in edible produce to allow for better monitoring and understanding of the environmental impact of PFAS on our food sources.

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## References

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<https://www.fda.gov/food/chemical-contaminants-food/analytical-results-testing-food-pfas-environmental-contamination>>
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## Appendix

Compound	PFAS group	Parent	Fragment	CV	CE	Internal standard	Fig 4 peak assignment
PFBA	carboxylate	213.0	169	8	5	<sup>13</sup> C-PFBA	1
PFPeA	carboxylate	262.9	218.9	5	5	<sup>13</sup> C <sub>6</sub> -PFPeA	2
PFHxA	carboxylate	312.9	268.9	16	6	<sup>13</sup> C <sub>5</sub> -PFHxA	3
			118.9	16	21		
PFHpA	carboxylate	362.9	318.9	14	8	<sup>13</sup> C <sub>4</sub> -PFHpA	4
			168.9	14	14		
PFOA	carboxylate	412.9	368.9	22	7	<sup>13</sup> C <sub>8</sub> -PFOA	5
			168.9	22	15		
PFNA	carboxylate	462.9	418.9	18	9	<sup>13</sup> C <sub>9</sub> -PFNA	6
			218.9	18	15		
PFDA	carboxylate	512.9	468.9	6	9	<sup>13</sup> C <sub>6</sub> -PFDA	7
			218.9	6	15		
PFUnDA	carboxylate	562.9	518.9	8	8	<sup>13</sup> C <sub>7</sub> -PFUnDA	8
			268.9	8	14		
PFDoDA	carboxylate	612.9	568.9	12	12	<sup>13</sup> C-PFDoDA	9
			168.9	12	21		
PFTriDA	carboxylate	662.9	168.9	14	22	<sup>13</sup> C-PFDoDA	10
			218.9	14	20		
PFTreDA	carboxylate	712.9	218.9	14	22	PFTreDA	11
			168.9	14	20		
PFBS	sulfonate	298.9	80.1	7	27	<sup>13</sup> C <sub>3</sub> -PFBS	12
			99.1	7	27		
PFPeS	sulfonate	348.9	79.9	32	31	<sup>13</sup> C <sub>3</sub> -PFHxS	13
			98.9	32	25		
PFHxS	sulfonate	398.9	80.1	38	35	<sup>13</sup> C <sub>3</sub> -PFHxS	14
			99.1	38	29		
PFHpS	sulfonate	448.9	79.9	16	34	<sup>13</sup> C <sub>8</sub> -PFOS	15
			98.9	16	34		
PFOS	sulfonate	498.9	79.9	30	42	<sup>13</sup> C <sub>8</sub> -PFOS	16
			98.9	30	40		
PFNS	sulfonate	548.9	80.1	24	40	<sup>13</sup> C <sub>8</sub> -PFOS	17
			99.1	24	36		
PFDS	sulfonate	598.9	80.1	46	46	<sup>13</sup> C <sub>8</sub> -PFOS	18
			99.1	46	46		
GenX (HFPO-DA)	emerging	285.0	169	5	7	<sup>13</sup> C <sub>3</sub> -GenX	19
			GenX	119	5		
ADONA	emerging	376.9	251	12	10	<sup>13</sup> C <sub>3</sub> -GenX	20
			377.3	84.9	12		
9Cl-PF3ONS	emerging	531.0	351	14	22	<sup>13</sup> C <sub>8</sub> -PFOS	21
			82.9	14	20		
11Cl-PF3OUdS	emerging	631.0	450.9	16	26	<sup>13</sup> C <sub>9</sub> -PFNA	22
			631.2	82.9	16		
4:2 FTS	precursor	326.9	306.9	42	18	<sup>13</sup> C <sub>2</sub> -4:2 FTS	23
			327.3	80.9	42		
6:2 FTS	precursor	427.0	406.9	12	22	<sup>13</sup> C <sub>2</sub> -6:2 FTS	25
			427.3	80.1	12		
8:2 FTS	precursor	526.9	506.9	28	26	<sup>13</sup> C <sub>2</sub> -8:2 FTS	27
			527.3	80.9	28		
FBSA	precursor	297.9	78	25	25	<sup>13</sup> C <sub>4</sub> -PFHpA	24
			118.9	25	15		



Compound	PFAS group	Parent	Fragment	CV	CE	Internal standard	Fig 4 peak assignment
FHxSA	precursor	398.0	78.1	30	25	<sup>13</sup> C <sub>4</sub> -PFHpA	26
			169	30	25		
FOSA	precursor	498.0	77.9	40	29	<sup>13</sup> C <sub>8</sub> -FOSA	30
N-MeFOSAA	precursor	569.9	418.9	36	15	D <sub>3</sub> -N-MeFOSAA	28
			168.9	36	27		
N-EtFOSAA	precursor	584.0	418.9	34	15	D <sub>3</sub> -N-EtFOSAA	29
			525.9	34	18		
<sup>13</sup> C-PFBA	-	217	172	7	8	<sup>13</sup> C <sub>2</sub> -PFOA	-
<sup>13</sup> C <sub>5</sub> -PFPeA	-	268	223	11	7	<sup>13</sup> C <sub>2</sub> -PFOA	-
<sup>13</sup> C <sub>5</sub> -PFHxA	-	318	273	10	6	<sup>13</sup> C <sub>2</sub> -PFOA	-
			120	10	18		
<sup>13</sup> C <sub>4</sub> -PFHpA	-	367	322	16	7	<sup>13</sup> C <sub>2</sub> -PFOA	-
			172	16	15		
<sup>13</sup> C <sub>8</sub> -PFOA	-	421	376	6	8	<sup>13</sup> C <sub>2</sub> -PFOA	-
			172	6	16		
<sup>13</sup> C <sub>9</sub> -PFNA	-	472	172	7	18	<sup>13</sup> C <sub>2</sub> -PFOA	-
			223	7	18		
<sup>13</sup> C <sub>6</sub> -PFDA	-	519	473.9	25	7	<sup>13</sup> C-PFDA	-
			219	25	13		
<sup>13</sup> C <sub>7</sub> -PFUnDA	-	569.9	524.9	9	8	<sup>13</sup> C-PFDA	-
			273.9	9	14		
<sup>13</sup> C-PFDoDA	-	615	569.9	23	10	<sup>13</sup> C-PFDA	-
			168.9	23	22		
<sup>13</sup> C <sub>2</sub> -PFTrEaDA	-	715	168.9	18	25	<sup>13</sup> C-PFDA	-
			219	18	25		
<sup>13</sup> C <sub>3</sub> -PFBS	-	301.9	80.1	34	28	<sup>13</sup> C-PFOS	-
			99.1	34	24		
<sup>13</sup> C <sub>3</sub> -PFHxS	-	402	80.1	13	38	<sup>13</sup> C-PFOS	-
			99.1	13	30		
<sup>13</sup> C <sub>8</sub> -PFOS	-	507	80.1	36	34	<sup>13</sup> C-PFOS	-
			99.1	36	34		
<sup>13</sup> C <sub>8</sub> -FOSA	-	506	77.9	13	28	<sup>13</sup> C <sub>2</sub> -PFOA	-
D <sub>5</sub> -N-EtFOSAA	-	589	418.9	24	17	<sup>13</sup> C <sub>2</sub> -PFOA	-
			482.9	24	13		
D <sub>3</sub> -N-MeFOSAA	-	573	418.9	17	18	<sup>13</sup> C <sub>2</sub> -PFOA	-
			515	17	18		
<sup>13</sup> C <sub>2</sub> -4:2 FTS	-	329	309	14	18	<sup>13</sup> C-PFOS	-
			80.9	14	21		
<sup>13</sup> C <sub>2</sub> -6:2 FTS	-	429	409	48	21	<sup>13</sup> C-PFOS	-
			80.9	48	27		
<sup>13</sup> C <sub>2</sub> -8:2 FTS	-	529	509	20	27	<sup>13</sup> C-PFOS	-
			80.9	20	37		
<sup>13</sup> C <sub>3</sub> -GenX	-	287	169	5	12	<sup>13</sup> C <sub>2</sub> -PFOA	-
			119	5	12		
<sup>13</sup> C <sub>2</sub> -PFOA	-	415	370	10	10	-	-
			169	10	15		
<sup>13</sup> C-PFOS	-	503	80.1	5	40	-	-
			99.1	5	40		
<sup>13</sup> C-PFDA	-	515	470	20	10	-	-
			219	20	15		

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720007333, Revised August 2023



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