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Analysis of Ultrashort-chain PFAS in Tomatoes Using a Luna Polar Pesticides HPLC Column

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Overview

Food safety remains a top priority for both industry and consumers, with accurate contaminant quantification being essential to protect public health. Among these contaminants, per- and polyfluoroalkyl substances (PFAS) have garnered increasing attention due to their environmental persistence and links to serious health concerns, including cancer and immune system suppression. Short- and ultra-short-chain (USC) PFAS, in particular, pose unique challenges due to their high mobility and bioavailability in water-rich environments, making them more likely to enter the food chain especially through high-moisture foods.

While most current studies on USC-PFAS focus on simple matrices like drinking water, limited work has addressed their quantification in complex food samples. This study presents a targeted analytical method for detecting USC-PFAS (C1–C4, including TFA, PFPrA, PFBA, PFMeS, PFEtS, PFPrS, PFBS, and DFA) in tomato for their high-water content and potential interferences from pigments and hydrophilic compounds. The newly developed chromatographic method improves retention and sensitivity for these analytes, offering a robust solution for PFAS detection in complex food matrices.

Sample Preparation

The extraction of tomato samples was conducted following the ion pair protocol described in EURL-POPs guidance [1].

Step	Description
1	Sample (2 g) + STD + ILIS in 50mL centrifuge tube
2	Add 2 mL Tetrabutylammoniumhydrogensulfate solution (0.5 M) + 4 mL Sodium carbonate solution (0.25 M) + 20 mL MTBE (Methyl tert butylether) + shaking (25 min)
3	Centrifugation (2500 x g, RT, 10 min)
4	Solvent evaporation to dryness at 40 °C
5	Dissolve residue in 300 µL MeOH/1% formic acid (2:1:v:v) in ultrasonic bath
6	Transfer supernatant in PP vial
7	UHPLC-MS/MS

LC Conditions

Column: Luna Polar Pesticides 3µm
Dimensions: 150 x 2.1mm
Delay Column: Luna Polar Pesticides 3µm
Dimensions: 100 x 2.1mm
Part No.: 00F-4798-AN
Mobile Phase: A (0.3% (v/v) HCOOH in H_2O)
B (0.3% (v/v) HCOOH in ACN)

Gradient:

Time (min)	% B
0	10
1	10
2	50
13	90
14	100
15	100
16	10
22	10

Flow Rate: 0.400mL/min
Injection Volume: 10µL
Temperature: 45°C
LC System: SCIEX ExionLC™ AC System
Detection: Electrospray Ionization Tandem Mass Spectrometer (ESI-MS/MS)
Detector: QTRAP® 6500+

MS/MS Conditions

Ion Source: IonDrive® Turbo V
Polarity: Negative
Source Temperature: 400°C
GS1: 35psi
GS2: 45psi
CUR: 35psi
IS Voltage: -4000V

Table 1. MRM Transitions. *Quantifier ions

Analyte	Internal Standard	Q1 Mass (Da)	Q3 Mass (Da)	Dwell Time (msec)	DP (V)	EP (V)	CE (V)	CXP (V)
DFA_1*	13C_TFA	95.0	50.8	80	-50	-14	-19	-11
DFA_2	13C_TFA	95.0	95.0	80	-50	-14	-5	-11
TFA_1*	13C_TFA	113.0	68.9	80	-1	-3	-15	-6
TFA_2	13C_TFA	113.0	19.0	80	-1	-3	-40	-10
PFPrA_1*	13C3_PFPrA	163.0	118.9	80	-4	-14	-15	-10
PFPrA_2	13C3_PFPrA	163.0	19.0	80	-4	-14	-40	-10
PFBA_1*	13C3_PFBA	212.9	168.9	110	-23	-7	-14	-18
PFBA_2	13C3_PFBA	212.9	212.9	110	-23	-7	-5	-18
PFMES_1*	13C4_PFBS	149.0	80.0	80	-20	-5	-31	-7
PFMES_2	13C4_PFBS	149.0	98.9	80	-20	-5	-31	-7
PFEtS_1*	13C4_PFBS	199.0	80.0	80	-42	-7	-32	-14
PFEtS_2	13C4_PFBS	199.0	98.9	80	-42	-7	-32	-14
PFPrS_1*	13C4_PFBS	249.0	80.0	90	-17	-5	-65	-15
PFPrS_2	13C4_PFBS	249.0	98.9	90	-17	-5	-35	-15
PFBS_1*	13C4_PFBS	299.0	80.0	130	-50	-12	-73	-9
PFBS_2	13C4_PFBS	299.0	98.9	130	-50	-12	-40	-9

Have questions or want more details on implementing this method? We would love to help!
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Figure 1. Chromatographic separation of ultrashort chain PFAS standards at 0.750 µg/kg at 0.750 µg/kg for DFA, TFA, and PFPrA and 0.075 µg/kg for PFMeS, PFEtS, PFPrS, PFBA, and PFBS

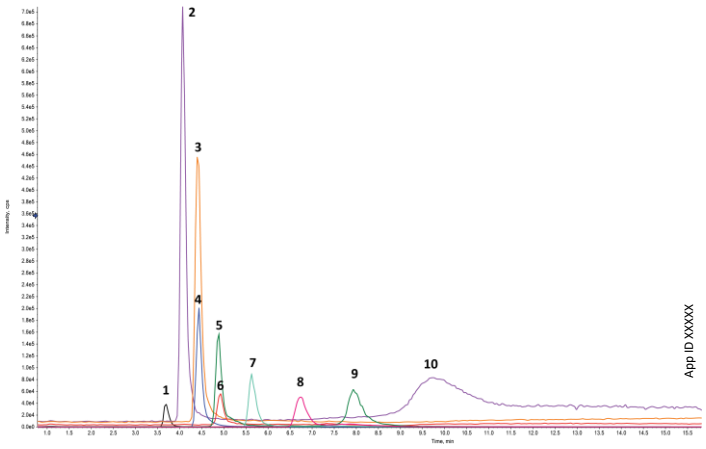


Table 2. USC-PFAS paek's identification and respective retention time.

Peak No.	Retention Time	Analytes
1	3.69	DFA
2	4.07	TFA
2	4.41	PFPrA
4	4.43	PFBA
5	4.88	PFMeS
6	4.92	PFEtS
7	5.64	PFPrS
8	6.73	PFBS
9	8.95	PFEtS from delay column
10	9.85	TFA from delay column

Figure 2. Chromatographic separation of TFA standard at 0.750 µg/kg (MRM transition 112.9/68.9).

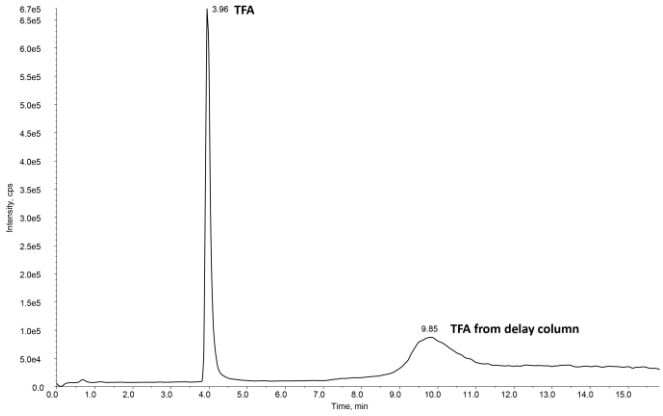


Table 3. TFA peak's identification and respective retention time.

Peak No.	Retention Time	Analytes
1	4.07	TFA
2	9.85	TFA from delay column

Figure 3. Chromatographic separation of USC-PFAS standard in tomatoes matrix at 0.750 µg/kg for DFA, TFA, and PFPrA and 0.075 µg/kg for PFMeS, PFEtS, PFPrS, PFBA, and PFBS

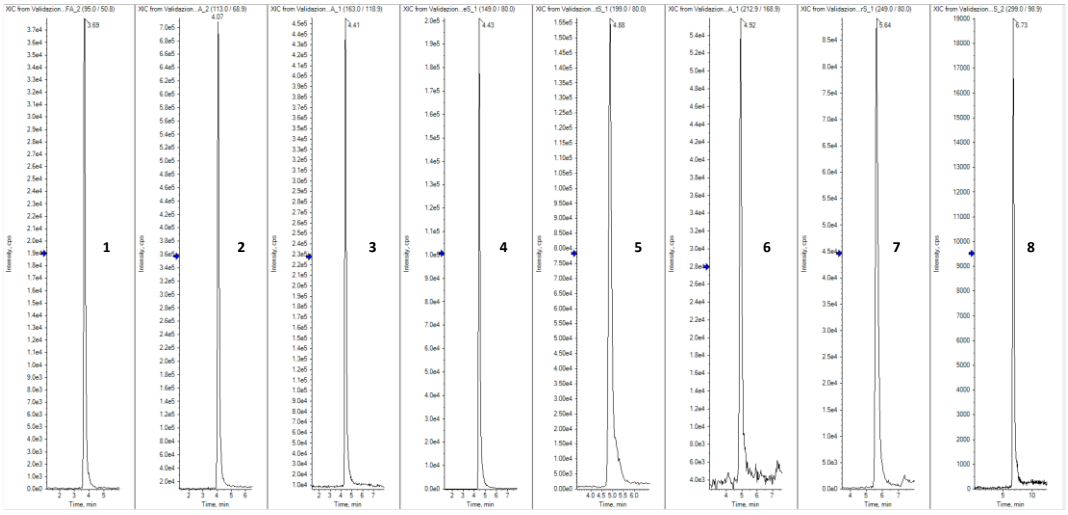


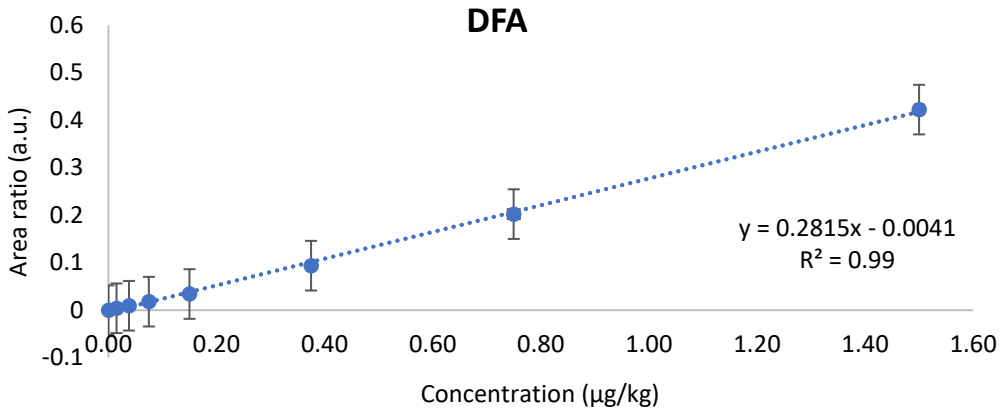
Table 4. Peak identification on the chromatogram

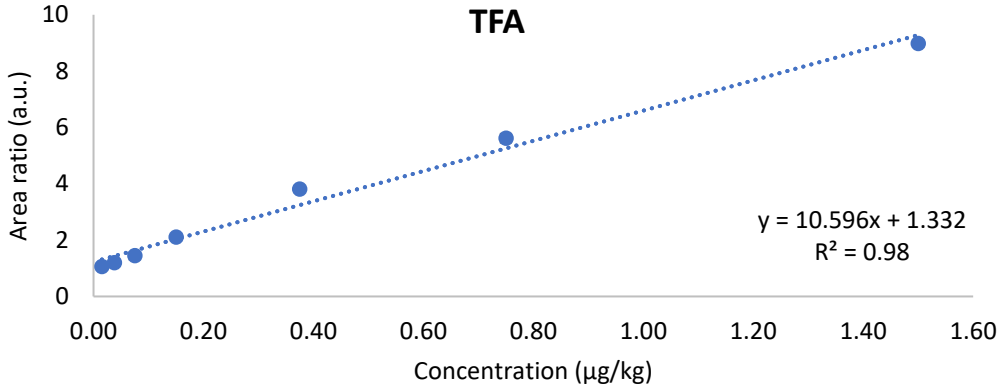
Peak No.	Analytes
1	DFA
2	TFA
2	PFPrA
4	PFBA
5	PFMeS
6	PFEtS
7	PFPrS
8	PFBS

Calibration Curves

An isotope dilution method was employed to construct four distinct calibration curves, with seven concentration points each. Three of these curves were created in a solvent, while one involved spiking an extracted matrix. For DFA, TFA, and PFPrA, calibration standards were prepared at concentrations ranging from 0 to 1.500 µg/kg (specifically, 0, 0.015, 0.038, 0.075, 0.150, 0.375, 0.750, and 1.500 µg/kg). Each of these standards contained a constant 0.375 µg/kg of isotopically labeled internal standards (specifically, ^{13}C-TFA and $^{13}C_3$-PFPrA). Similarly, for PFMeS, PFEtS, PFPrS, PFBA, and PFBS, calibration levels were established from 0 to 0.150 µg/kg (0, 0.002, 0.008, 0.015, 0.038, 0.075, 0.113, and 0.150 µg/kg), with an internal standard concentration of 0.075 µg/kg for $^{13}C_3$-PFBA and $^{13}C_4$-PFBS.

Figure 4 . Calibration curves, average of five





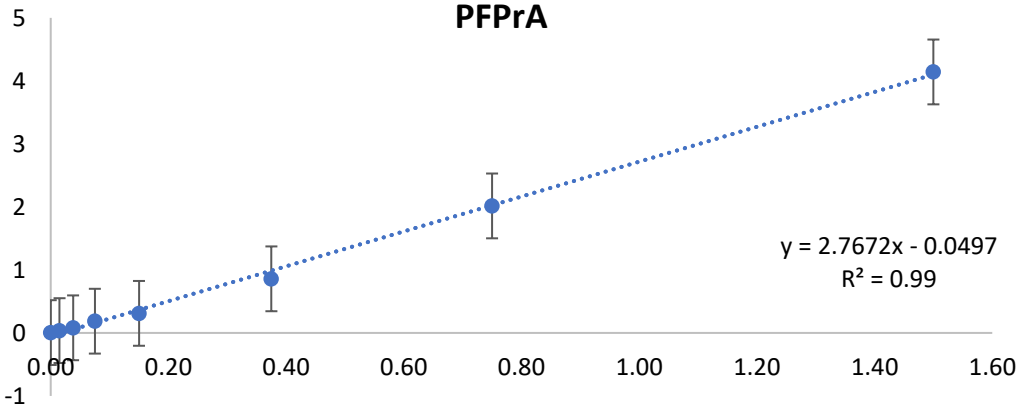
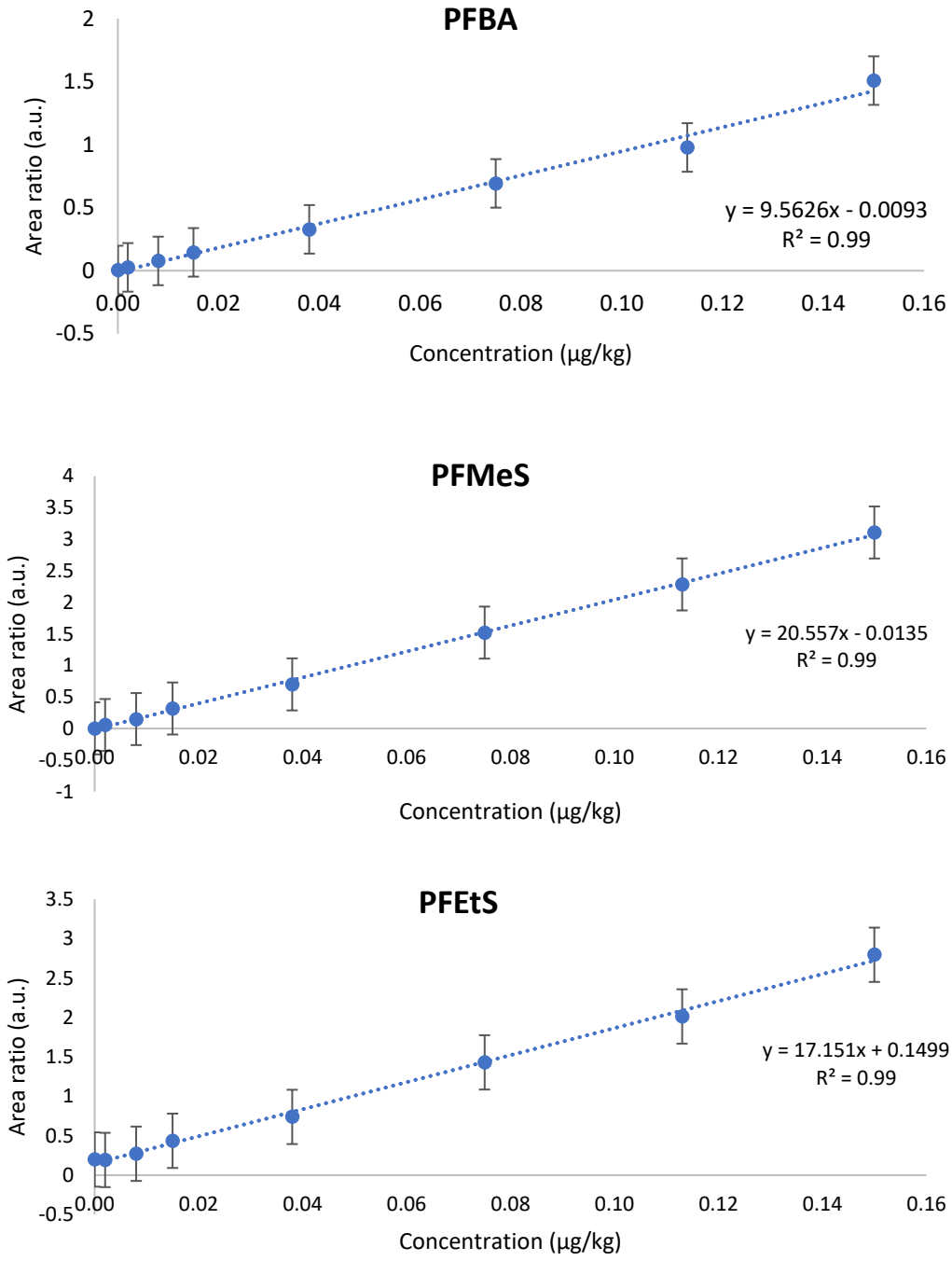
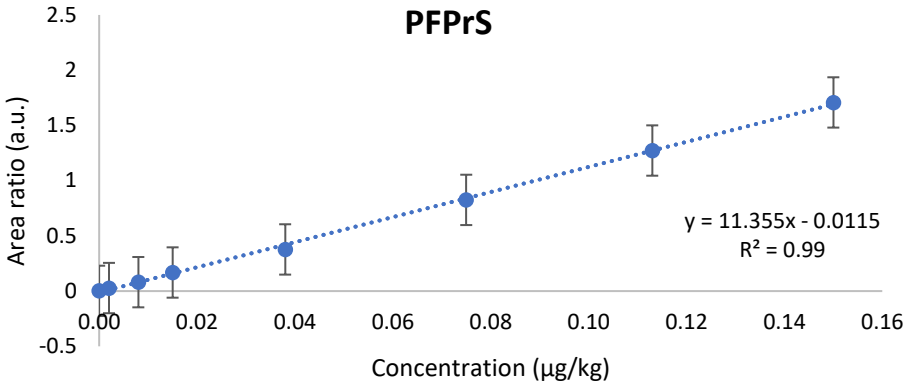
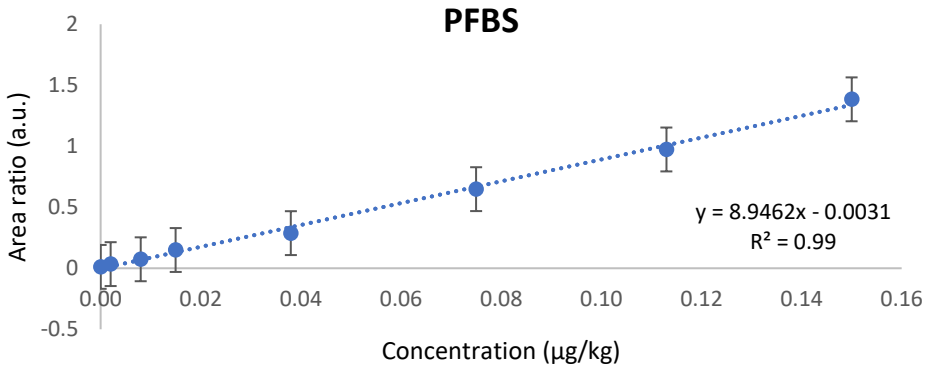


Figure 4 . Calibration curves, average of five



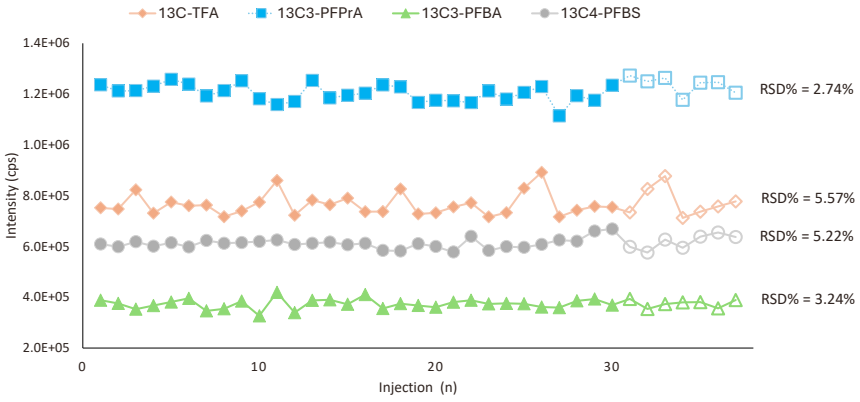




Matrix Effect and Method Precision

Method precision and Matrix effect: The method's precision was assessed by integrating the areas of labeled PFAS in calibration curves across 37 injections from different vials, both in solvent and matrix. The RSD% for ^{13}C-TFA, $^{13}C_3$-PFPrA, $^{13}C_3$-PFBA, and $^{13}C_4$-PFBS were 5.57, 2.74, 5.22, and 3.24, respectively **(Figure 5).**

Figure 5. Area of labeled USC-PFAS variation along 37 injection of standard in solvent and fortified matrix



Conclusion and Acknowledgment

This study presents a novel method for detecting ultra-short-chain PFAS in complex food matrices such as tomato products. It introduces a polar analytical and delay column setup that minimizes system interferences thanks to a huge delay of TFA contamination coming from system. This effect significantly improves sensitivity, reliability, and robustness.

In addition, the method shows excellent linearity ($R^2 > 0.99$) for all analytes, including TFA, in both solvent and matrix.

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References

[1] C. Schiavone, F. Romaniello, P. Brizio, M. Suman, A. M. Rossi, C. Portesi, Reliable and sensitive ultra-short chain per- and polyfluorinated alkyl substances (PFAS) analysis in food: a polar reverse-phase chromatography approach, Journal of Chromatography A, 2025, 466332, ISSN 0021-9673, https://doi.org/10.1016/j.chroma.2025.466332

[2] European Union Reference Laboratory for POPs (EURL), Section 6.3.2, Module 1B: Solid-liquid extraction based on ion-pair extraction, in Annex V2.0 of the Guidance Document on Analytical Parameters for the Determination of Per- and Polyfluoroalkyl Substances (PFAS) in Food and Feed (issued 10 September 2024)

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