Method development and validation of a serum and urine method for the analysis of ultra-short chain PFAS in humans

Kevin Stroski[1,*], Marta Malevic[1], Francesca Cappelli[1], Adrian Covaci[1]

1. Toxicology Centre, University of Antwerpen

University of Antwerp | Toxicological Centre

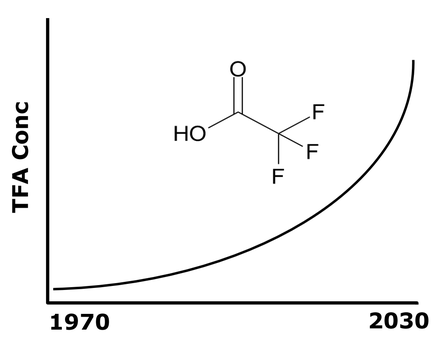
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BACKGROUND



Ultra-short chain (USC) PFAS, especially **trifluoroacetic acid (TFA)**, are consistently detected in the environment[1], food[2], animals[3], and water[4] around the world.



Environmental concentrations of TFA are expected to continue to rise due to **industrial runoff**, use of **F-gases** in air conditioning, and **degradation of CF_3** containing compounds[1].

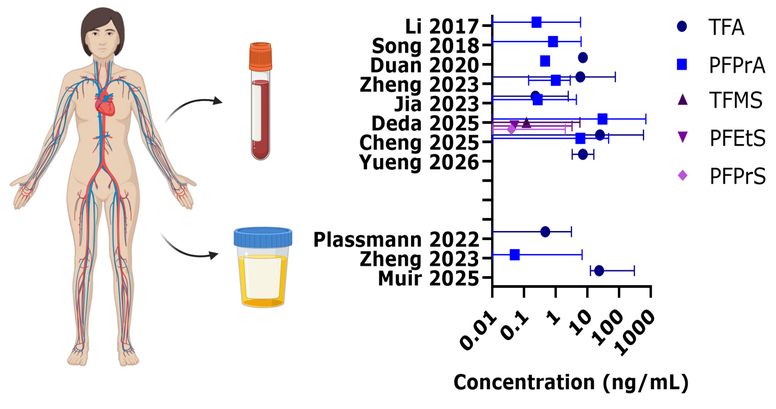


Figure 1. Mean (min/max) USC-PFAS concentrations in human serum (top) and urine (bottom) from literature

Data coverage on USC-PFAS concentrations in humans is **limited and variable**. Further, the connection between urine and serum is not well understood.

OBJECTIVES

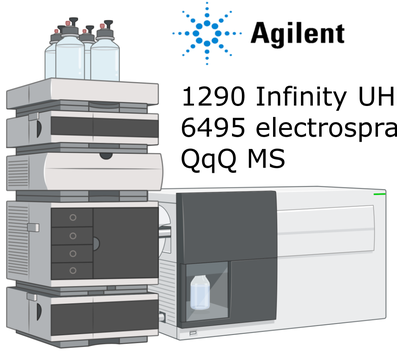
Develop a **robust and universal LC-MS/MS method** for the analysis of USC-PFAS.

Develop and validate extraction methods for **serum** and **urine** analysis of USC-PFAS

Estimate USC-PFAS concentrations in **paired urine and serum samples** and identify trends between the matrices.

Make **recommendations** for USC-PFAS analysis and long-term human monitoring studies.

INSTRUMENTATION



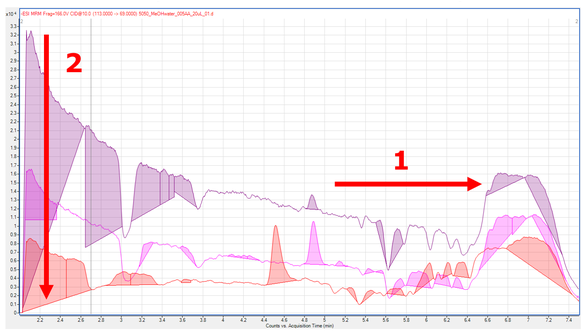
Agilent

1290 Infinity UHPLC
6495 electrospray QqQ MS

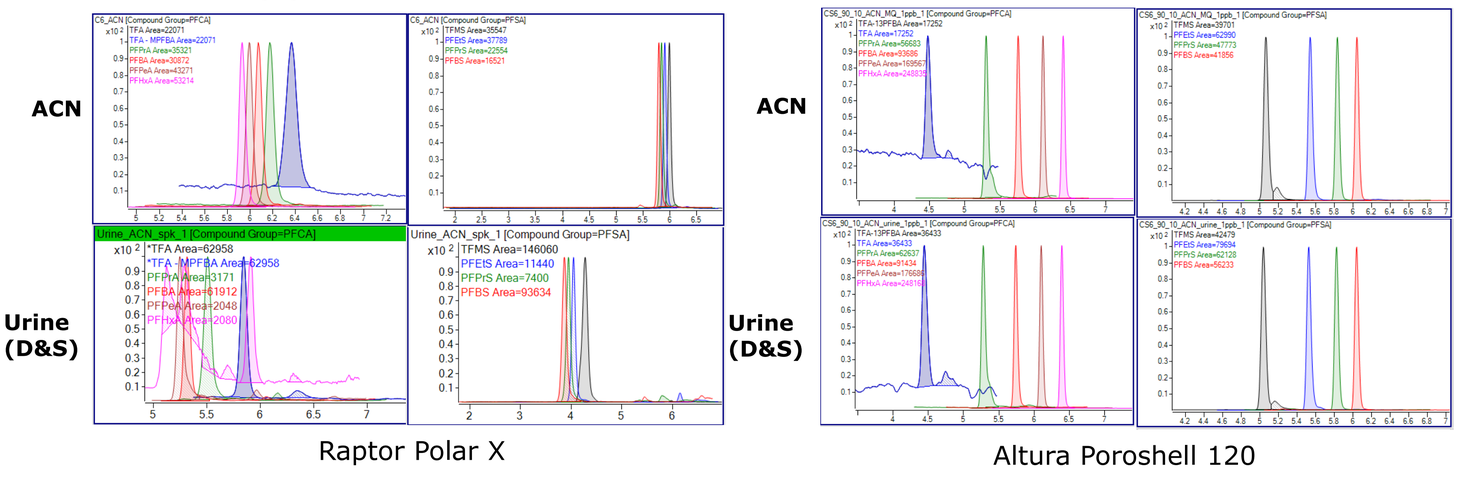
Compounds: TFA, PFPrA, PFBA, PFPeA, PFHxA, PFMOAA, TFMS, PFEtS, PRPrS, PFBS

TFA Contamination Solutions

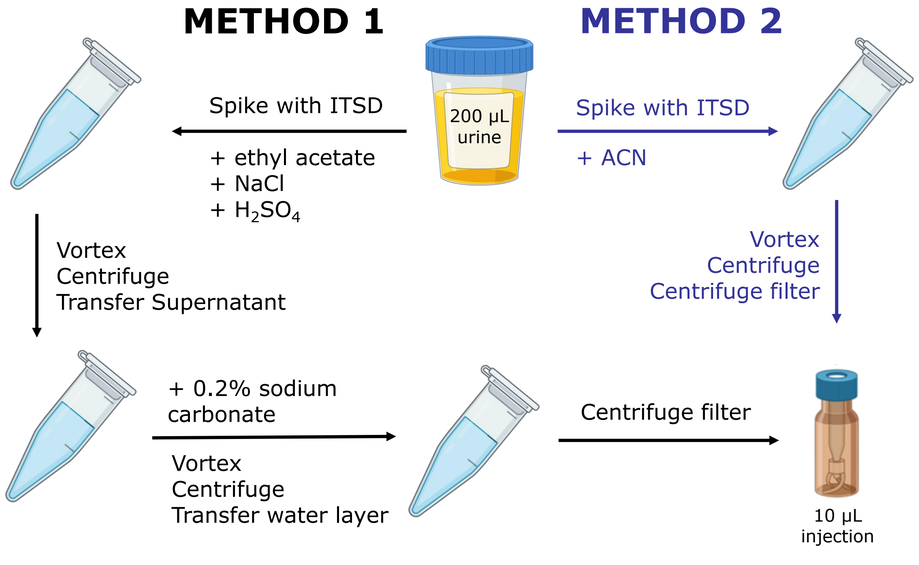
1. D**elay column** (Agilent PFC Delay) installed to delay background TFA found in solvents and instrument
2. Baseline significantly increased following **CheckTune**. Required **36 hours** of flushing to reduce. Avoid tunes prior to USC-PFAS analysis

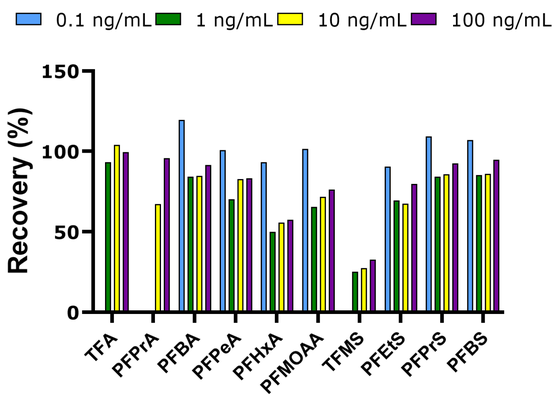


COLUMN TESTS



EXTRACTION





Method 1 validation (left) was successful for 6/10 compounds, however had high contamination of TFA and PFPrA.

Method 2 validation (right) successful for all compounds, though PFPrA contamination still present above 1 ng/mL.

URINE

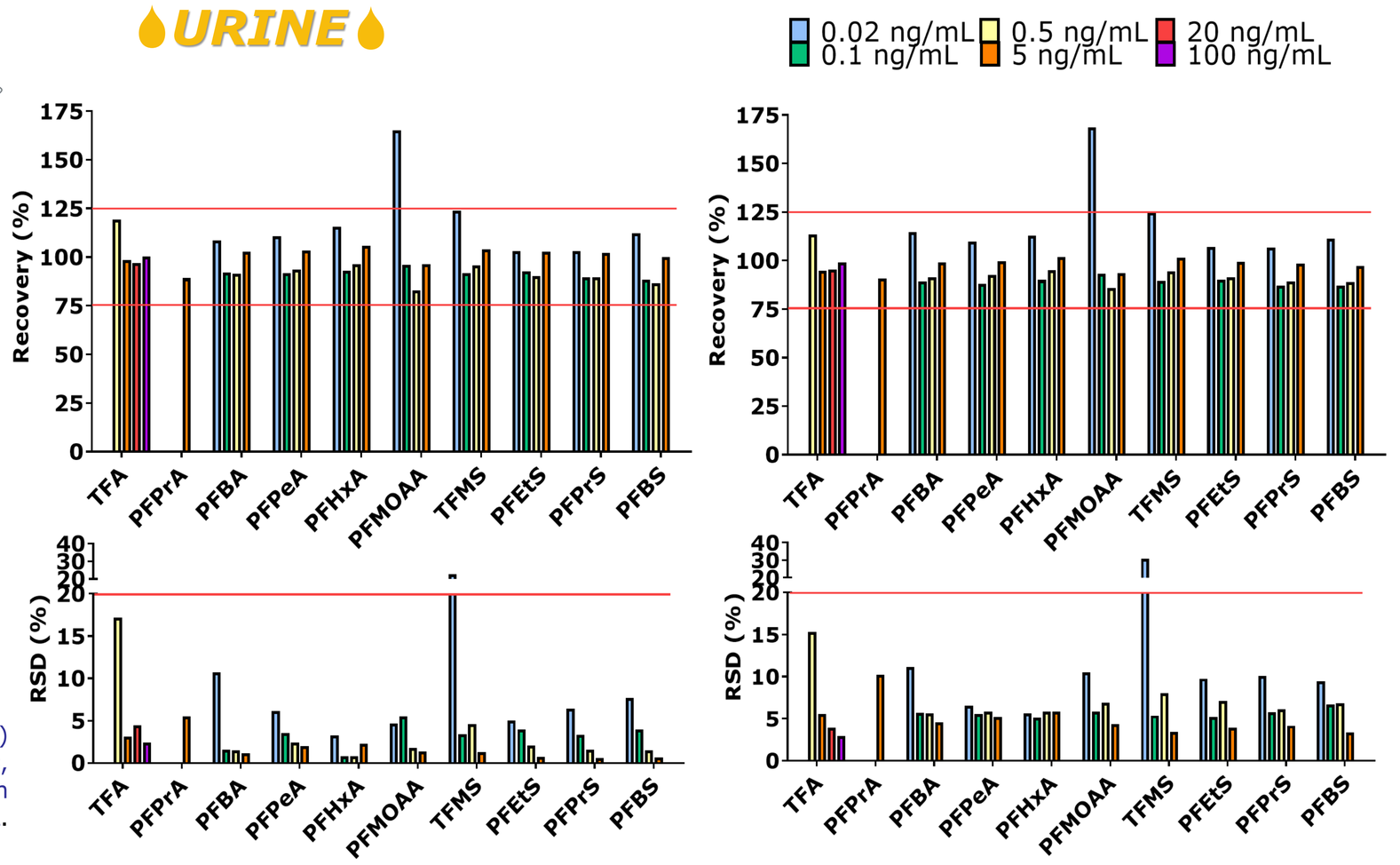
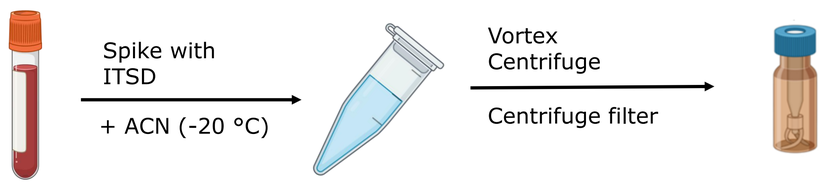


Table 1. Limits of detection and quantification (ng/mL) for urine extraction method 2

	TFA	PFPrA	PFBA	PFPeA	PFHxA	PFMOAA	TFMS	PFEtS	PFPrS	PFBS
LOD	0.213	1.407	0.002	0.002	0.003	0.005	0.002	0.010	0.002	0.003
LOQ	0.5	5	0.02	0.02	0.02	0.1	0.1	0.02	0.02	0.02

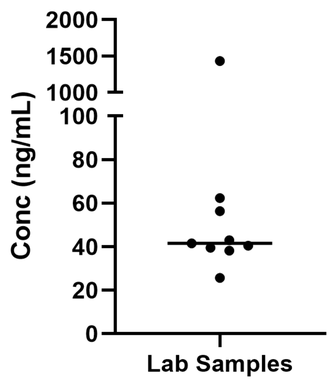
SERUM



COMING SOON

Serum method validation planned for upcoming month

RESULTS



Urine samples from 10 volunteers in the lab collected and analyzed

Median concentration of TFA was 41 ng/mL while one outlier measured above 1400 ng/mL.

Post-mortem urine and serum samples being collected to analyze relationship between urine and serum

CONCLUSIONS

Agilent Altura Poroshell 120 analytical column works well for USC-PFAS, uses reverse phase gradient and can include long-chain PFAS

TFA contamination in solvents and instrument MUST be accounted for and managed throughout each extraction and run

Batch LOQs required for TFA and PFPrA due to ongoing contamination

Urine extraction method 1 worked well for PFCAs but not PFSAs, however may be useful in some applications (e.g. TOP assay)

Urine extraction method 2 successfully validated across 4 spiking levels (except for PFPrA below 1 ng/mL)

Highly variable urine concentrations detected in both laboratory volunteer samples highlighting need for broader analysis.

LITERATURE

1. Hanson et al. 2024 *Environ Toxicol Chem*, 43: 2091-2093
2. Sapozhnkova 2026. *J Agric Food Chem,* 3;74(21):16668-16673
3. Junque et al. 2026. *Environ Pollut,* 390: 127515
4. Cappelli et al. 2024. *Environ Research,* 260: 119753
5. Selke et al. 2026. *Environ Int,* 215, 110504