From Complex Matrix to Clear Peaks: Sensitive and robust PFAS quantification in Milk

Nick van Eijndhoven, Bas van Dijk, Ruben Kause, Stefan van Leeuwen

Introduction

Per- and polyfluoroalkyl substances (PFASs) are synthetic chemicals known for their persistence and potential health risks even at low concentrations. Due to their widespread use, PFASs are commonly found in the environment and can accumulate in the food chain, including animal-derived products like milk. For adequate exposure and risk assessment, sensitive and reliable analytical methods are essential for accurate detection. However, PFAS analysis in milk is challenging due to matrix interferences like fats and proteins. Other studies on milk analysis lacked proper removal of matrix components, sensitivity, robustness and required a matrix-matched calibration. This study presents a sensitive, robust and easy to handle LC-MS/MS method for the determination of 22 PFASs in five different milk types using solvent-based calibration methods.

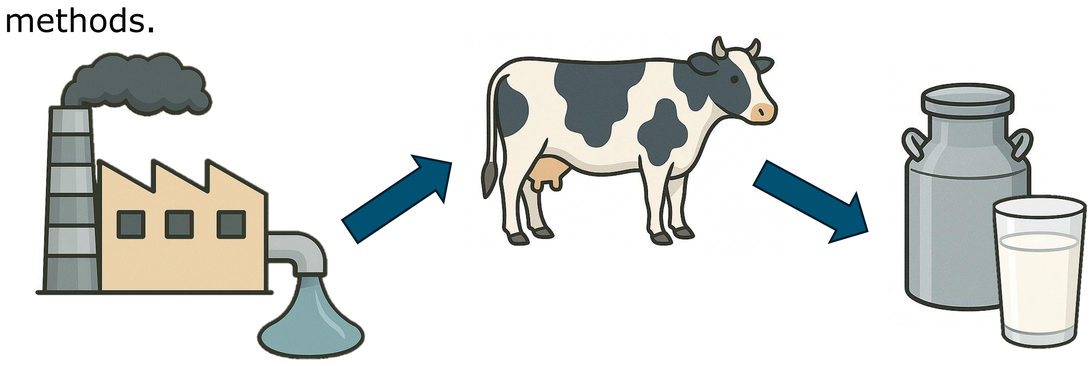

Figure 1. Schematic representation of the PFAS contamination pathway from industrial discharges to cows, leading to residues in milk.

Methods

The PFASs included in this study were carboxylic acids (PFCAs), sulfonic acids (PFSAs), telomer sulfonates (FTSs), HFPO-DA (also known as Gen X), DONA, 9CL-PF3ONS, and 11CL-PF3OUdS. The two-phase extraction method was developed using acetonitrile, combined with 5.0g of QuEChERS salts (4.0g $MgSO_4$ + 1.0g NaCl) and formic acid. The extract was further cleaned using heptane to remove co-extracted fats, followed by a mixed-mode solid-phase extraction (Strata-X-AW SPE). Analysis was performed using the Sciex7500 LC-MS/MS system. The method has been validated on five different milk types: whole, semi-skimmed, skimmed, long-life, and protein-enriched milk.

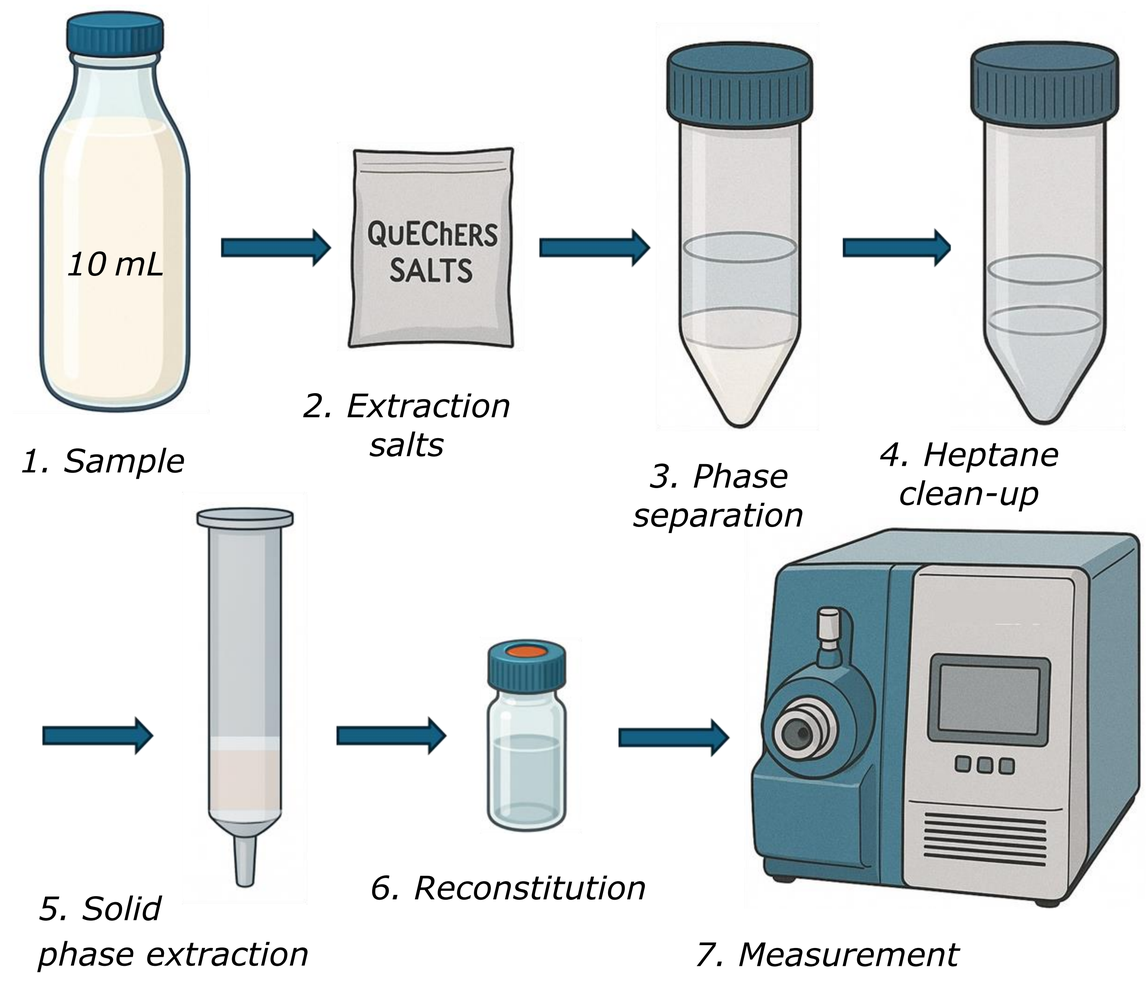

Figure 2. Schematic overview of the PFAS extraction from milk using QuEChERS salts and acetonitrile, followed by clean-up with heptane and mixed-mode SPE (Strata-X-AW), and final analysis by LC-MS/MS.

Results

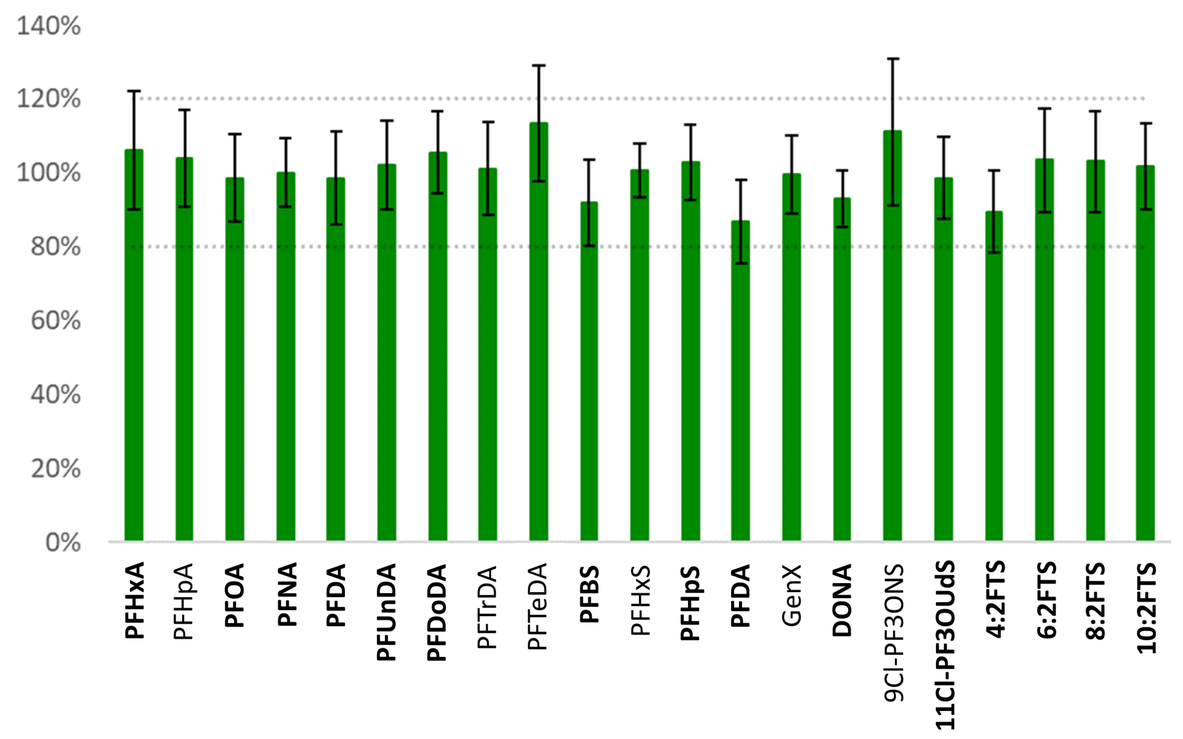

Figure 3. Average trueness at the limit of quantification (LOQ) level for various PFAS compounds. Compounds with a LOQ of < 2.5 ng/L are shown in bold, while those with a LOQ of < 5.0 ng/L are not bolded. Bars represent the mean trueness values, with error bars indicating the standard deviation. The dotted lines at 80% and 120% indicate the accepted trueness range, as defined by the compliance testing limits in Commission Implementing Regulation (EU) 2022/1428.

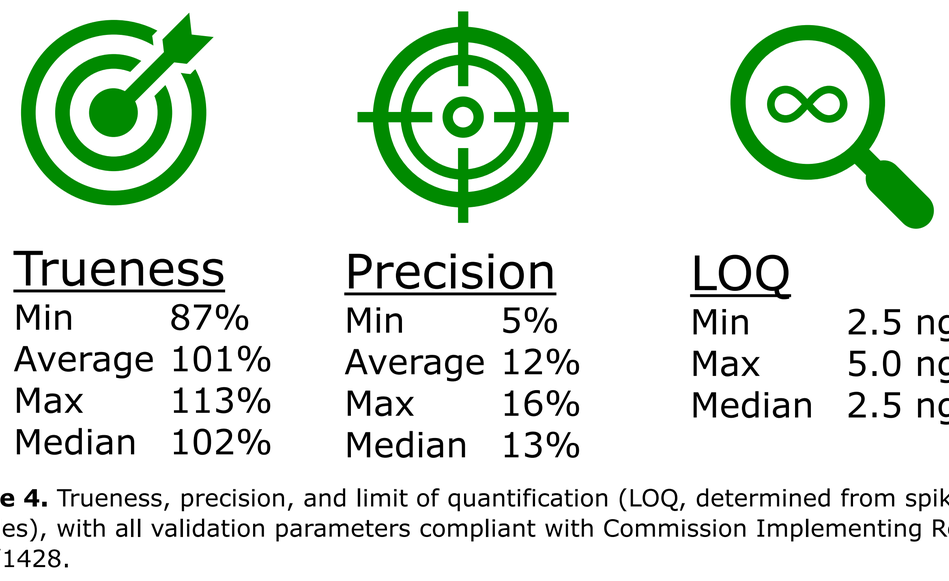

Figure 4. Trueness, precision, and limit of quantification (LOQ, determined from spiked milk samples), with all validation parameters compliant with Commission Implementing Regulation (EU) 2022/1428.

Conclusions

- A robust multi-step extraction method was developed for reliable PFAS analysis different milk samples.
- Validation in milk showed successful extraction of 22 PFASs across five milk types, with LOQs as low as 2,5 ng/L; being ¼ of the recommended LOQs (EU 2022/1431).
- Method performance meets EU criteria (EU 2022/1428), with trueness between 87–113% and RSD_r (precision) < 20%.

Wageningen Food Safety & Research
Contact: Nick.vaneijndhoven@wur.nl
T +31 (0)317 48 02 56, M +31 (0)6 24 353 772
www.wur.nl/WFSR

Acknowledgements

The Dutch Ministry of Agriculture, Fisheries, Food Security and Nature is gratefully acknowledged for financial support (project WOT-02-001-017).