Rapid LC-MS/MS method for monitoring bio-relevant levels of per-and polyfluoroalkyl substances (PFAS) in serum

Using MRM on the SCIEX QTRAP 6500+ System

RUO-MKT-11-13069-A

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Introduction

PFAS

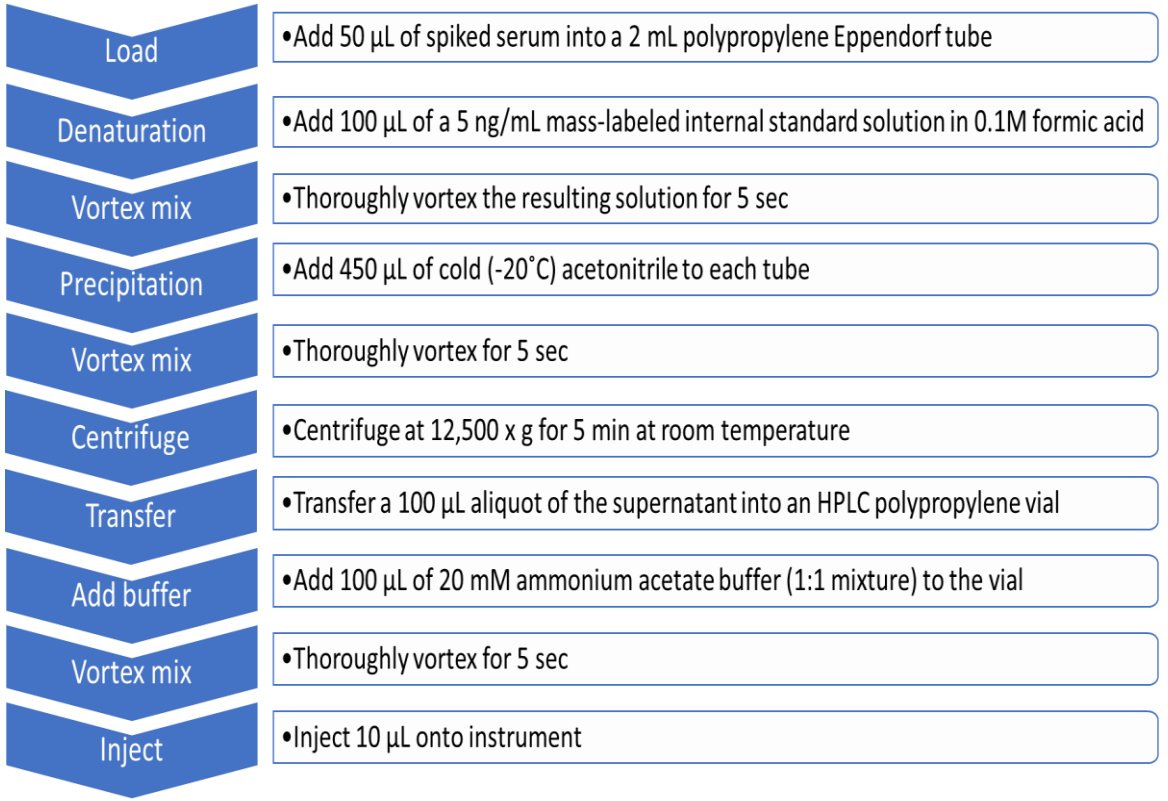
- Per- and polyfluoroalkyl substances (PFAS) are pervasive compounds used in a variety of industrial applications and found in a wide range of consumer products such as cookware, stain repellant, flame-retardant and coatings
- Bioaccumulation of PFAS in the human body resulting from environmental exposure is a growing public health concern
- Given the prevalence and ubiquitous nature of PFAS in the environment and every-day consumer products (including our drinking water supply), there is a critical need to develop quantitative tools capable of accurately and precisely detecting low-levels of PFAS in biological fluids to inform the extent of their bioaccumulation and overall impact on the human body
- Here a quantitative workflow for the analysis of PFAS in serum was developed using the SCIEX QTRAP 6500+ System
- This targeted screening workflow provides a fast analytical method capable of accurately quantifying sub-nanogram per mL levels of PFAS in the human body
  - Sub ng/mL detection for a panel of 22 PFAS extracted from serum samples
  - Excellent linearity across a wide linear dynamic range (0.5 to 100 ng/mL)
  - Method adaptable for high-throughput biomonitoring studies aimed at determining the potential toxic effects of PFAS bioaccumulation associated with human exposure

Extraction of PFAS from serum samples

PFAS EXTRACTION PROCEDURE

- 50 µL of spiked serum were placed in a 2 mL polypropylene Eppendorf tube
- 100 µL of a 5 ng/mL mass-labeled internal solution in 0.1M formic acid was added
- 450 µL of cold (-20˚C) acetonitrile was added to the tube
- The tube was thoroughly vortexed for 5 seconds
- The tube was centrifuged at 12,000 x g for 5 minutes at room temperature
- 100 µL of the supernatant was transferred into an HPLC polypropylene vial
- 100 µL of 20 mM ammonium acetate buffer (1:1 mixture) was added to the HPLC vial
- The HPLC vial was thoroughly vortexed for 5 seconds
- 10 µL were injected onto the instrument

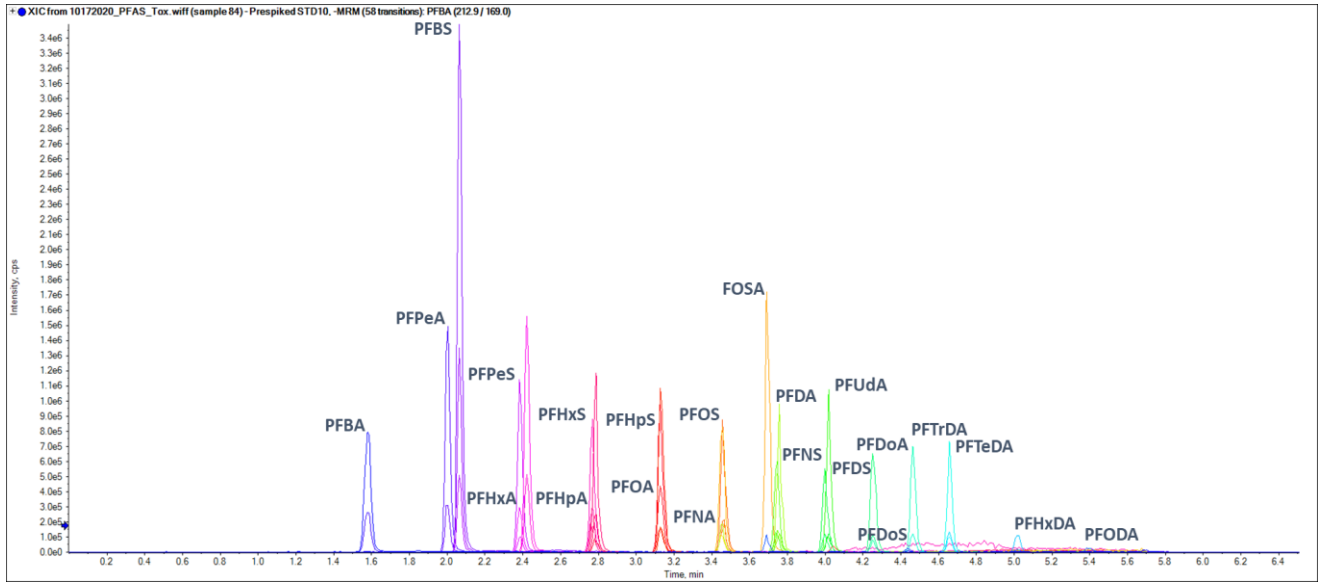
10-Step protein precipitation procedure for serum samples



Chromatography

PFAS

- UHPLC separation was performed on a Phenomenex Gemini® C18 column (50 x 2 mm, 3 µm, 00B-4439-B0) at 25ºC on a SCIEX ExionLC™ AC System
- Mobile phase A – ammonium acetate and water
- Mobile phase B – formic acid and methanol
- The injection volume was 10 µL
- The flow rate was 0.6 mL/min
- Total LC runtime was 8 minutes



Chromatographic profile of the 22 PFAS in a 6.5 minute runtime

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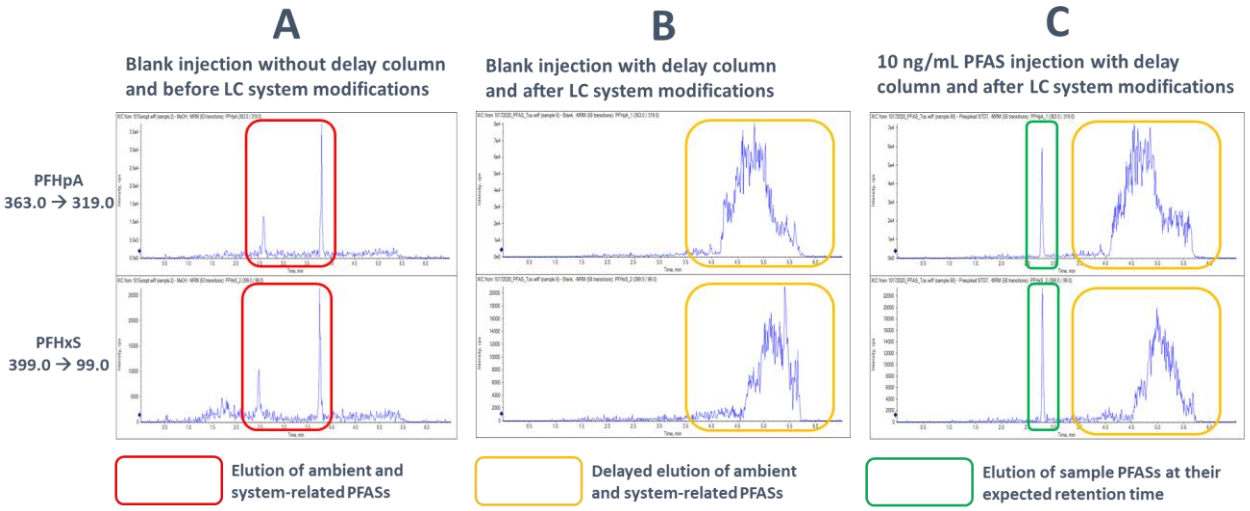
Delay column

PFAS

- A Phenomenex Luna C18(2) column (30 x 2 mm, 5 µm, 00A-4252-Y0) was installed between the pump mixing chamber and the analytical column used for separation
- This additional column served as a delay column to isolate PFAS contamination leaching from the LC system components and minimize the risk of system-related PFAS interfering with real signals from the sample during the analytical run
- The addition of the delay column and the modifications made to the LC system components together minimized the impact of system-related PFAS contamination and ensured the analytical integrity of this quantitative workflow



Benefits of using a delay column for PFAS analysis

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Data acquisition

SCIEX QTRAP 6500+ SYSTEM

- Scheduled MRM was used to collect two transitions per analyte in negative ion mode
- SCIEX QTRAP 6500+ System with Analyst Software
- High acquisition rate for fast chromatography
- Scheduled MRM Algorithm was used to optimize data sampling across each peak and maximize the dwell times used, ensuring reliable integration, quantification and confirmation for each of the PFAS

Data processing

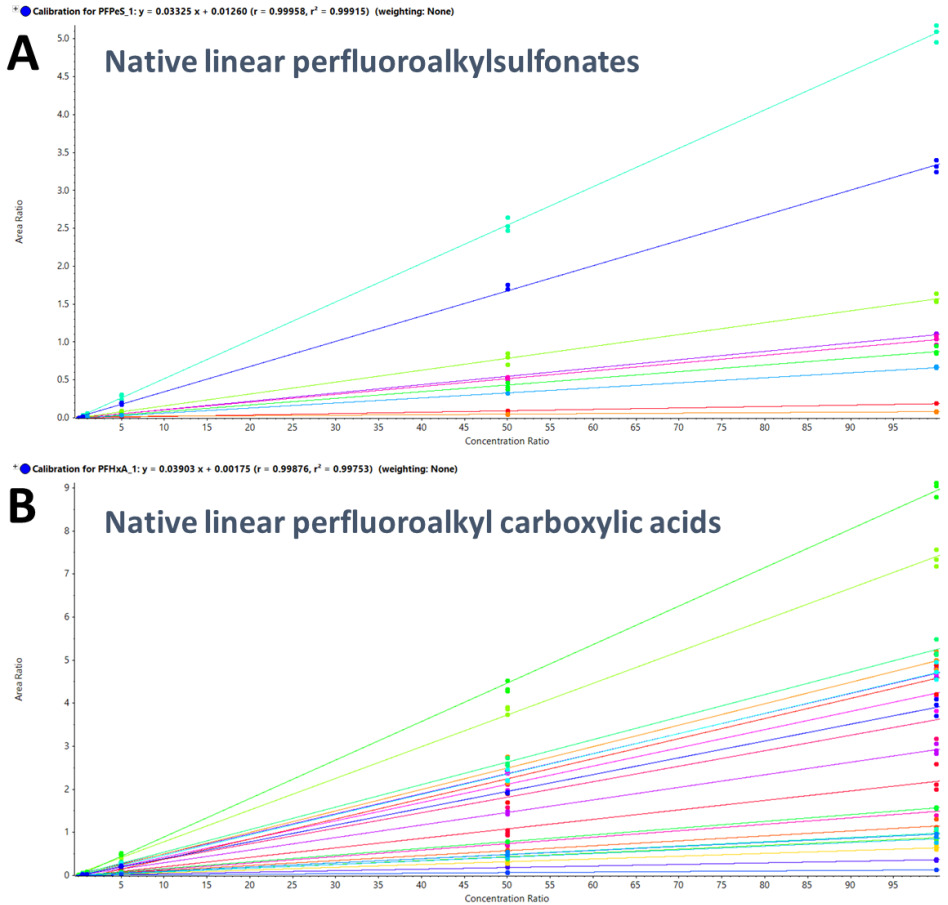
SCIEX OS 2.0

- Data processing was performed using SCIEX OS Software
- Detection and integration of the peaks from the background was achieved using the MQ4 algorithm
- Quantitative analysis was performed in the Analytics module of the software where calibration curves, concentration calculations, assay precision and accuracy statistics were automatically generated

Accurate and reliable quantification

CALIBRATION CURVES FOR THE 22 PFAS

- Excellent linearity with R^2 value > 0.99



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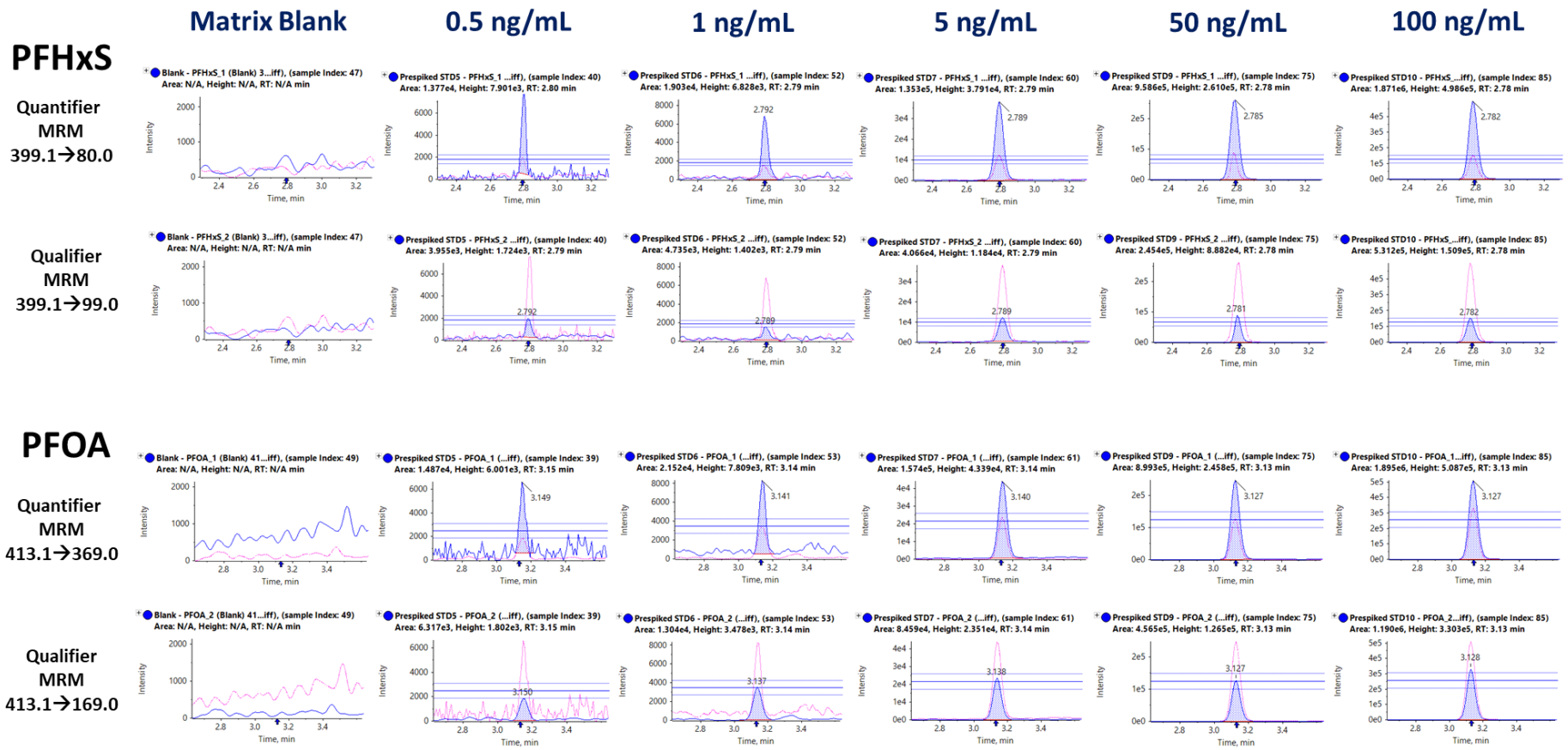
Accurate and reliable quantification

XICS FROM PFAS EXTRACTED FROM SERUM SAMPLES

- Excellent quantification performance



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Accurate and sensitive detection

LOD AND LLOQ

- High sensitivity detection of PFHxS

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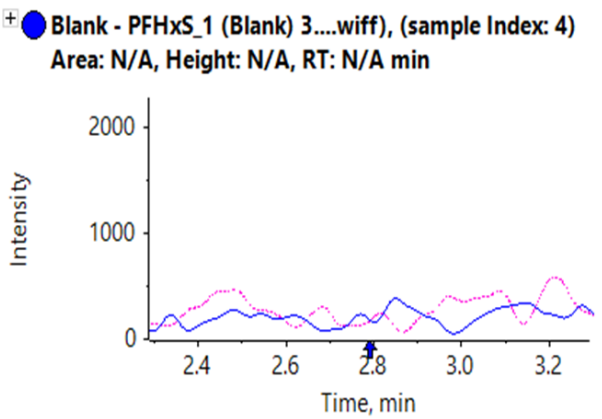
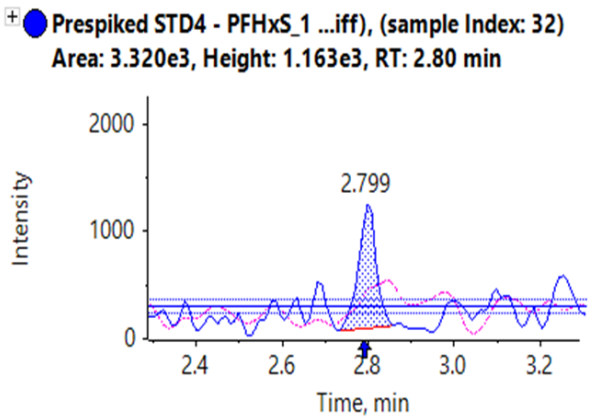
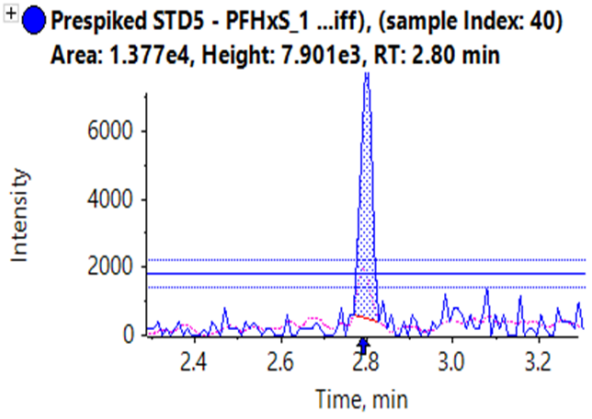


LOD
0.1 ng/mL



LLOQ
0.5 ng/mL



Conclusions

- A robust and sensitive workflow for the detection of PFAS in serum samples using the SCIEX QTRAP 6500+ System was successfully developed
- The addition of a delay column and the modifications made to the LC system components reduced the risk of system-related PFA interferences
- The combination of a simple sample preparation procedure with a fast LC separation enabled accurate and sensitive detection of 22 PFAS down to sub ng/mL levels
- The assay showed excellent reproducibility, precision, accuracy and linearity, with an LLOQ of 0.5 ng/mL, LOD of 0.1 ng/mL and an R^2 of greater than 0.99 for the vast majority of the PFAS in the panel with the exception of PFBS and PFODA
- The presented workflow is readily adaptable for high-throughput toxicology investigations aimed at determining the extent of PFAS bio-accumulation and its broader impact on human health

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Thank you

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