Seminal plasma as a noninvasive sensitive diagnostic matrix for the evaluation of PFAS exposure in firefighters

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Introduction

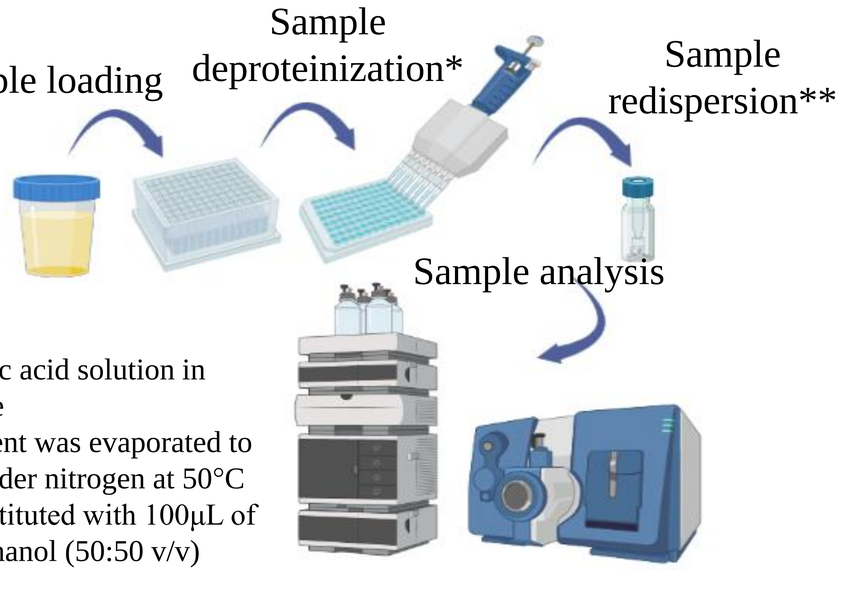
- Per- and polyfluoroalkyl substances (PFASs) are a group of synthetic chemicals with water resistant properties primarily used in variety of products such as carpets, furniture, paints, fabrics etc.
- PFAS exposures have been associated with adverse health outcomes, including cancer and fertility issues
- Firefighters have elevated concentrations of certain PFASs compare to non-firefighters
- Firefighters are exposed to PFAS through the combustion of PFAS-containing products such as furniture and carpet and through firefighting gear and firefighting foams that contain these compounds
- Seminal plasma is an alternative matrix to blood to monitor for PFAS exposure

Aims and Objectives

- To optimize sample preparation method for selective extraction of 15 PFASs from seminal fluid
- To develop and validate a method for simultaneous quantification of PFASs using liquid-chromatography tandem mass spectrometry (LC-MS/MS)

Materials and methods

- **A 96 well plate sample preparation method:**



- *1% formic acid solution in acetonitrile
- **The eluent was evaporated to dryness under nitrogen at 50°C and reconstituted with 100µL of water-methanol (50:50 v/v)

Figure 1: Workflow for detection of PFASs based on a 96-well plate sample preparation and analysis on LC-ESI-MS/MS.

- **LC-MSMS method:**

➢ Chromatographic conditions

Separation column: SYNERGI 4µ Fusion Max-RP 80Ä 100 mm x 2 mm (Phenomenex, USA)
Elution conditions: Gradient elution with a mixture of 5mM ammonium acetate and methanol; 400 µl/min flow rate

➢ MS/MS detection

QTrap 5500 (ABSciex, CA, USA) operating in ESI-negative mode

Results

- The method validation was performed based on FDA guidelines
- Validation criteria included limit of detection (LOD), limit of quantitation (LOQ), accuracy and precision
- Due to the lack of the certified reference standard material the validation criteria of the method were assessed in pool of in house spiked seminal samples (QC)

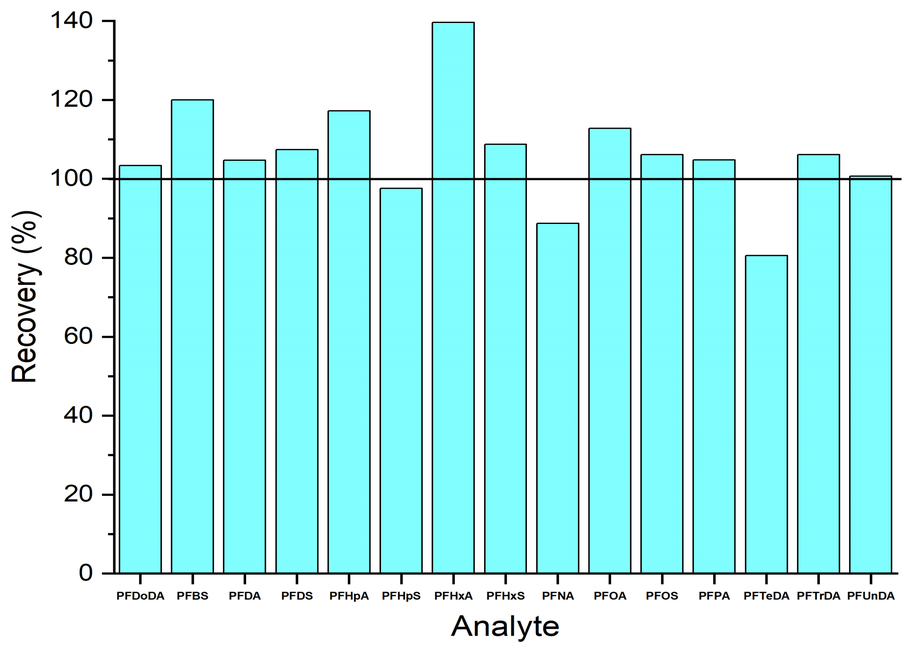


Figure 2: Precision and accuracy* of the extraction and quantification method

* The accuracy of the sample preparation method was evaluated as a percent recovery of the amount of target analyte added into the sample

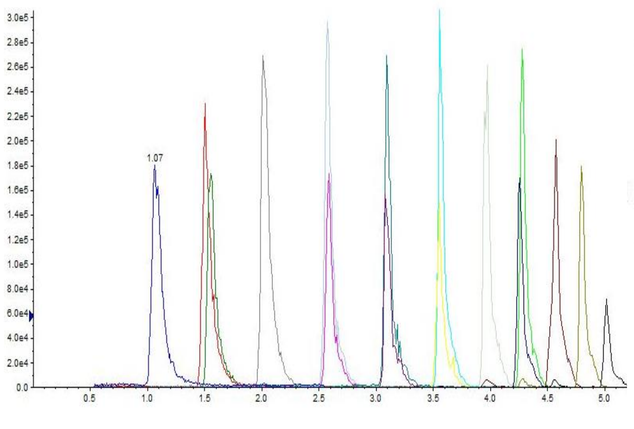


Figure 3: Representative LCMSMS chromatogram of PFAS mix calibration standard 1ng/mL

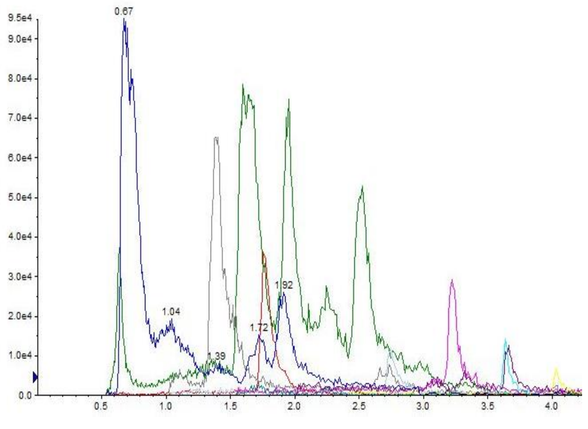


Figure 4: LCMSMS chromatogram of real sample prepared on 96 well plate

Conclusions

✓ Selective sample preparation method was optimized for detection of PFAS in seminal fluid samples by LC-MS/MS

✓ The precision and accuracy of the in house prepared QC samples showed that the method is robust and accurate

✓ Capability of being able to quantitively measure PFASs in seminal fluid in pg/mL concentration range can help to identify and establish their role in male infertility and serve as alternative method for their monitoring

Acknowledgement

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