Benchtop Matrix Assisted Laser Desorption Ionization Mass Spectrometry of PFAS for preliminary screening of environmental samples

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1. Introduction

- Per- and polyfluoroalkyl substances (PFAS) are persistent environmental contaminants which negatively impact human health and the environment.
- High concentrations of PFAS in test samples can cause contamination of sensitive LC-MS/MS instruments requiring extensive decontamination procedures, resulting in significant downtime and costly delays in sample processing.
- We present the development of a simple, cost-effective method for the rapid screening of PFAS samples using an entry-level linear benchtop MALDI-TOF mass spectrometer to identify samples containing high levels before they enter the analytical workflow.

System	:	MALDI-8030
Polarity	:	Negative
Mass Range	:	*m/z* 100-1000
Acquisition	:	980 shots @ 200Hz
Blanking	:	200
Pulsed Extraction	:	400

Fig. 1 Analysis Conditions for MALDI-8030 analysis of PFAS

2. Methods

PFOA and PFOS standards were purchased from Merck, UK. Stock solutions (1 ppm prepared in acetonitrile) were diluted in water to the required concentrations.

Samples were tested with a variety of MALDI matrices (CHCA, DHB, DHAP, DAN, NRM and TMGN), with norharmane (NRM) and 1,8-bis(tetramethylguanidino)-naphthalene (TMGN) identified for further investigation. Diluted samples were mixed with NRM (1 mg/mL) or TMGN (5 mg/mL) in 70:30 Acetonitrile:Water. Blank samples of UHP water were prepared to ensure no contamination was present. 1 µL of the mixture was spotted onto a FlexiMass-SR48 slide and the samples were analysed using a MALDI-8030 MALDI-TOF mass spectrometer (Shimadzu). Analysis conditions are shown in Fig 1. Peaks at *m/z* 314.2 and *m/z* 499.2 were monitored for PFOA and PFOS, respectively. $^{13}C_8$PFOS (*m/z* 507) was used as an internal standard for PFOS samples with a final sample concentration of 6.25 ng/mL (see Fig. 2).

MALDI-MSI was used to assess spot homogeneity and limits of detection were established.

Subsequently, PFHxA and PFBS standards were purchased from Merck, UK and analysed using the same method.

3. Results

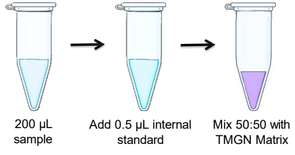

Fig. 2 Addition of internal standard and matrix is all that is required for analysis

- With both norharmane and TMGN, PFOS and PFOA were detected at concentrations suitable for preliminary screening to prevent contamination of LC-MS/MS systems[1]. MALDI-MSI spot imaging revealed the migration of PFAS to the edges of the spots during spotting/drying (Fig. 3). Use of an annular acquisition strategy for norharmane spots produced spectra with a higher signal to noise, as expected. Spots prepared with TMGN matrix showed a more homogeneous distribution and work was continued with this matrix (5 mg/mL in 70% acetonitrile).
- Limits of detection were established for PFOA, PFOS, PFHxA and PFBS standards at 5 ppb, 250 ppt, 200 ppt and 1 ppb respectively (Fig. 4).

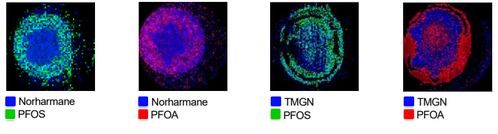

Fig. 3 MALDI-MSI Spot imaging of PFOS and PFOA in Norharmane & TMGN

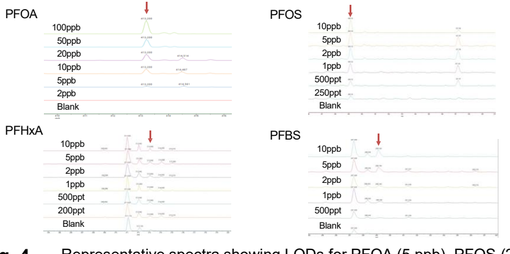

Fig. 4 Representative spectra showing LODs for PFOA (5 ppb), PFOS (250 ppt), PFHxA (200 ppt) and PFBS (1 ppb)

- Analysis of bottled water samples were chosen to be representative of samples received within a PFAS testing environment. A small number of samples showed low levels of possible PFAS present in the sample (Fig. 5).

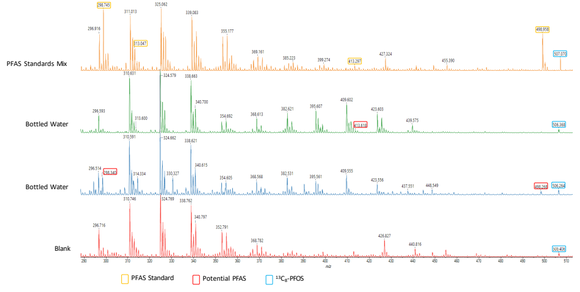

Fig. 5 Representative spectra showing analysis of bottled water alongside a mixed PFAS sample containing PFOS (50 ppb), PFOA (100 ppb), PFBS (50 ppb) and PFHxA (100 ppb) and a blank UHP water sample.

- To demonstrate how MALDI-TOF MS could be applied in a screening workflow, whole slide imaging was performed demonstrating a quick and simple method to visually identify potentially problematic samples.
- Whole slide imaging (250 µm spacing) of a full slide of standard samples (48 spots) with mixed concentration levels was completed in approx. 12 mins.
- Mixed concentration samples were spotted onto a standard 48 well FlexiMass-SR48 slide and also a 96 well FlexiFocus™ slide for imaging
- IMAGEREVEAL MS™ (Shimadzu, Japan) software was used to create the slide ion image using a red-green intensity colour bar to display high concentration samples in green and low concentrations in red (see Fig. 7).
- 100 ng/mL was chosen as a suitable cut off for identifying problematic PFOS samples. Setting the intensity scaling for 100 ng/mL samples as mid-range showing a mixture of red and green, allowed easy identification of high concentration samples as bright green. (See Fig. 7).

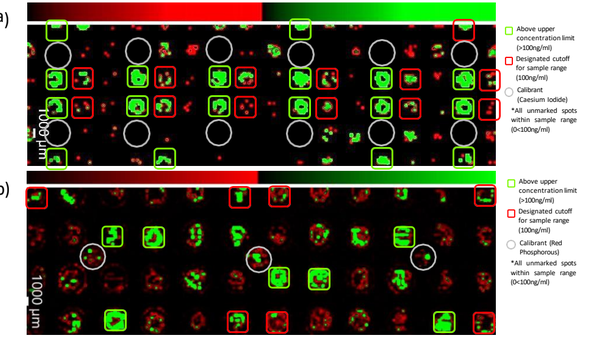

Fig. 7 Whole plate imaging as a rapid screening tool. In the examples shown, PFOS samples at 0, 1, 20, 50, 100 & 200 ng/mL were analysed after spotting onto a) a 96 well target or b) a 48 well target.

4. Conclusion

- We have shown, in principle, that it is possible to use MALDI-TOF mass spectrometry to implement a simple pre-screening method for aqueous samples to prevent widespread contamination in a PFAS monitoring laboratory. Using just a 200 µL sample and minimal preparation, a full slide of samples can be acquired in less than 15 minutes
- Spot imaging during method development highlighted the differences in homogeneity of PFOS and PFOA spots suggesting that multiple internal standards would be necessary to pursue semi-quantitative analysis.
- Whole slide imaging can provide quick results for multiple samples across a range of PFAS concentrations in a single run with easy visual identification of potentially problematic samples.
- As a linear MALDI-TOF method, these results do not confirm the presence of PFAS but may be used as an indicative result to modify further testing protocols. Confirmatory LC-MS/MS analysis is required to determine the presence and concentration of any PFAS.

References
1) Shimadzu Application News LCMS-151