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Quantification of PFAS in Plasma: a Faster Way to Monitor Human Exposure

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OVERVIEW

Purpose

- Optimization of an extraction process and LDTD-MS/MS analysis of PFAS in plasma for fast monitoring of PFAS exposure.

Method

- Liquid-Liquid Extraction (LLE).
- Samples dried and analyzed by LDTD-MS/MS.

Quantification

- Linearity: r^2> 0.99 over the calibration range.
- Inter-run accuracy, values between 88.5% and 112.6% were obtained and the precision results were lower than 10.6%CV.
- Samples analyzed with a runtime of 8 seconds using LDTD-MS/MS technique.

INTRODUCTION

Since the 1950s, Perfluoroalkyl and polyfluoroalkyl substances (PFAS) have been used in many consumer products and industrial processes. There are thousands of types of PFAS. The most common types and final products of degradation are PFOA (perfluorooctanoic acid) and PFOS (perfluorooctanoic sulfonic acid). They are widely detected in different environmental media and human blood. The National Health and Nutrition Examination Survey (NHANES) and European Food Safety Authority (EFSA) suggest guidelines with concentration ranges for PFAS exposition. The sum of the major PFAS between 2-20 ng/mL detected in human plasma can be a potential risk for adverse health effects which increase with concentrations greater than 20 ng/mL. To quickly monitor PFAS exposure levels in human plasma, LDTD-MS/MS is a useful tool.

LUXON Ionization Source:

The Luxon Ion Source (Figure 1) is the second-generation sample introduction and ionization source based on the LDTD technology for mass spectrometry. The Luxon Ion Source uses a Fiber-Coupled Laser Diode (Figure 2) to obtain unmatchable thermal uniformity giving more precision, accuracy and speed.
The process begins with dry samples which are rapidly evaporated using indirect heat. The thermally desorbed neutral molecules are carried into a corona discharge region. High-efficiency protonation and strong resistance to ionic suppression characterize this type of ionization and is the result of the absence of solvent and mobile phase. This thermal desorption process yields high-intensity molecular ion signal in less than 1 second sample-to-sample and allows working with very small volumes.

Figure 1 *Luxon Ion Source*

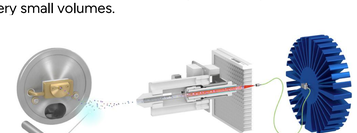

Figure 2 *Schematic of the Luxon Ion Source*

METHOD

Liquid-liquid extraction

In a borosilicate tube (16X100 mm), 400 µL of sample and 4 µL of internal standard solution are added. After mixing, 100 µL of HCl (1N), and 400 µL of BSA buffer are added for acidification and dilution of the sample. Samples are vortexed and 1600 µL of extraction solution (Acetonitrile/Cl_3CH: 1/1) are added. After mixing, and centrifugation steps, 800 µL of the bottom layer are transferred in a second borosilicate tube (12X75 mm). Samples are evaporated until dryness. 40 µL of reconstitution solution are added and vortexed. 5 µL of reconstituted sample are spotted on a LazWell™96 plate, and dried at 40°C.

Instrumentation

- Ion source: Phytronix Luxon Ion Source SH-960
- Mass spectrometer: LCMS-8060, Shimadzu

Luxon Parameters

Laser power pattern:
- Increase laser power to 100% in 6 sec
- Hold 1 second
- Carrier gas flow: 6 L/min (Air)

MS Parameters

- APCI (-)
- Corona Needle Voltage : -4 kV
- CID: 250 kPa
- Time: 20 msec

Table 1 *MRM transitions for LDTD-MS/MS*

	Transition	CE (eV)
PFOA	413.0 → 369.0	12
PFOA-M_8	421.0 → 376.0	12
PFOS	499.0 → 80.0	48
PFOS-M_8	507.0 → 80.0	48

RESULTS

Validation

Calibration curves range from 0.5 to 10 ng/mL. Calibration curve and a set of QCs are prepared in bovine serum albumin (20 mg/mL in PBS). Replicate extractions are deposited onto a LazWell™ plate and dried before analysis. The peak area against the internal standard (IS) ratio is used to normalize the signal.

Linearity

The calibration curves are plotted using the peak area ratio and the nominal concentration of standards. For the linearity test, the following acceptance criterion is used:

- Linear regression (r) must be ≥ 0.995

Table 2 shows the determination coefficient of six different runs.

Table 2 *Determination coefficient for curves*

	Determination coefficient (r^2)	
Run	PFOA	PFOS
1	0.99468	0.99278
2	0.99291	0.99274
3	0.99361	0.99047
4	0.99271	0.99346
5	0.99243	0.99331
6	0.99433	0.99297

RESULTS

Precision and Accuracy

Spiked sample solutions are used to validate the precision and accuracy of the method.
The following acceptance criteria were used:
- Each concentration must not exceed <20%CV.
- Each concentration must be within ±20%Bias.

Table 3 show the inter-run precision and accuracy results for PFOA and PFOS. %CV was below 20% and the accuracy within the 20%.

Table 3 *Inter-run Precision and Accuracy for PFOA and PFOS*

PFOA	QC-L	QC-M	QC-H
Conc (ng/mL)	1	4	8
N	18	18	18
Mean (ng/mL)	1.02	4.12	8.27
%CV	11.5	8.2	6.8
%Bias	1.5	3.1	3.3
PFOS	**QC-L**	**QC-M**	**QC-H**
Conc (ng/mL)	1	4	8
N	18	18	18
Mean (ng/mL)	1.05	4.26	8.31
%CV	11.5	6.0	7.9
%Bias	4.8	6.5	3.8

Stability

Wet stability of sample extracts:
Following the extraction, the sample extracts are kept at 4°C in closed containers protected from light. After 4 days, sample extracts are spotted on a LazWell™ plate, dried and analyzed. Precision and accuracy of QC samples are reported in **Table 4**. All the results are within the acceptable criteria range for 4 days at 4°C.

Dry stability of samples spotted on LazWell:
Extracted samples are spotted onto a LazWell™ plate, dried and kept at room temperature for 2 hours before analysis. The precision and accuracy results of QC samples are reported in **Table 4.** All the results are within the acceptable criteria range for 2 hours at room temperature.

Table 4 *Wet and dry stability for PFOA and PFOS*

PFOA	Wet stability (4 days / 4°C)			Dry stability (2 hours / RT)		
	QC-L	QC-M	QC-H	QC-L	QC-M	QC-H
Conc. (ng/mL)	1	4	8	1	4	8
N	3	3	3	3	3	3
Mean (ng/mL)	0.99	4.22	8.52	1.01	3.84	7.90
%CV	16.2	7.7	5.8	16.3	6.2	7.2
%Bias	-1.0	5.6	6.5	1.4	-4.0	-1.3
PFOS	**Wet stability (4 days / 4°C)**			**Dry stability (2 hours / RT)**		
	QC-L	QC-M	QC-H	QC-L	QC-M	QC-H
Conc. (ng/mL)	1	4	8	1	4	8
N	3	3	3	3	3	3
Mean (ng/mL)	1.03	4.26	8.40	1.02	3.82	8.02
%CV	14.3	4.3	8.1	13.5	3.0	3.7
%Bias	3.0	6.6	4.9	2.0	-4.6	0.2

Cross-validation study

Real patient plasmas have been tested with this method to correlate with results obtained by traditional LC-MS/MS method. **Figure 3** shows a correlation greater than 0.95 between both technologies.

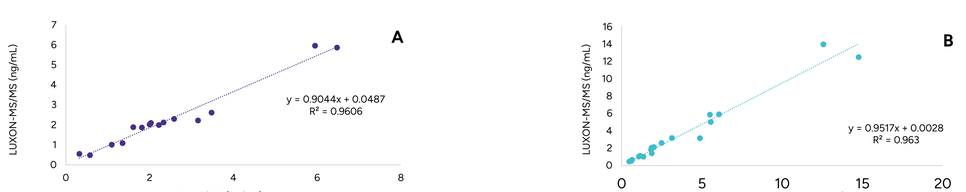

Figure 3 *Correlation evaluation for **A**) PFOA and **B**) PFOS (LC-MS/MS vs LDTD-MS/MS).*

CONCLUSION

- **Efficient Liquid-Liquid Extraction (LLE) is used to extract PFOA, and PFOS in plasma samples.**
- **High-throughput analysis using LDTD-MS/MS.**
- **Accuracy, precision, and stability within the acceptance criteria.**
- **Sample-to-sample analysis of 10 seconds.**

COI Disclosure: I have a financial relationship with Phytronix Technologies as a salaried employee.