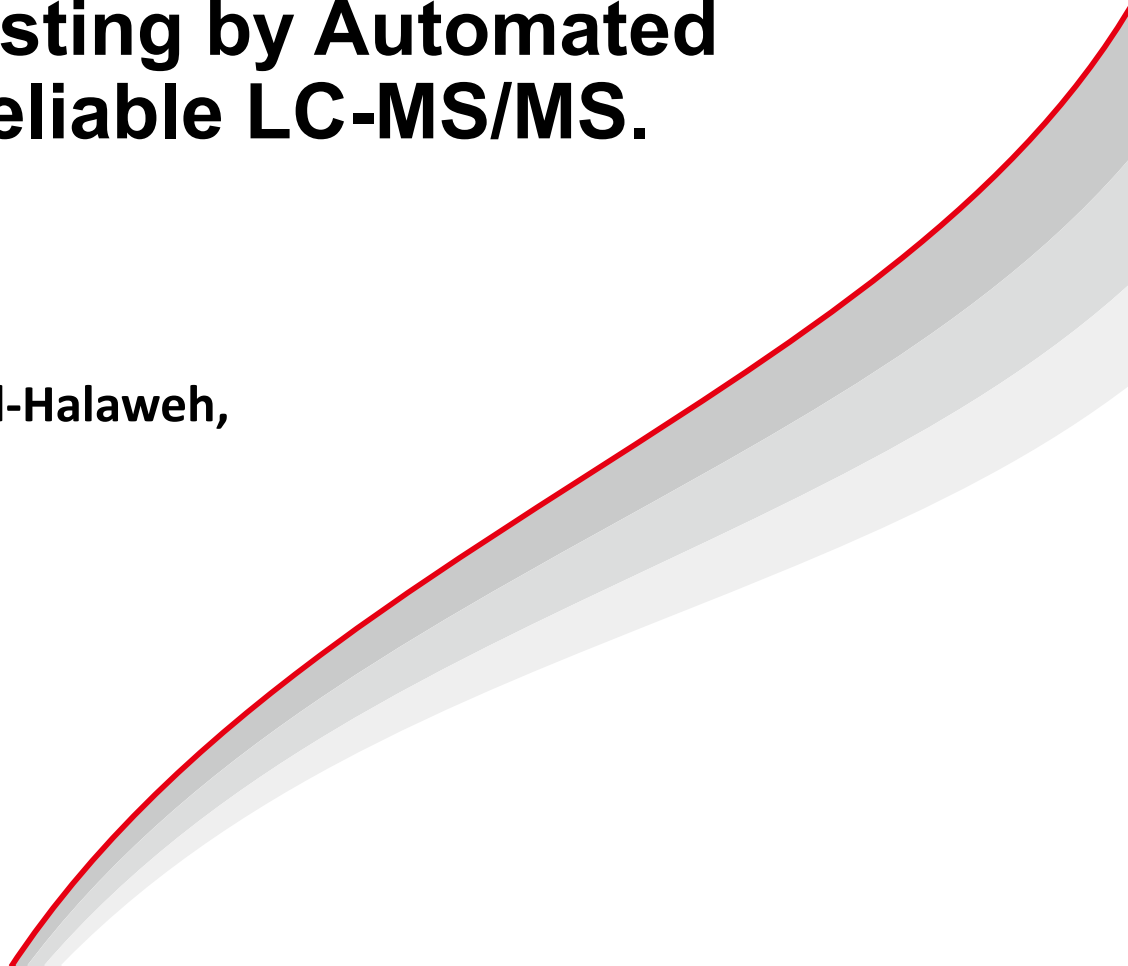


# **From EPA 1633 to 1633A: The Evolution of PFAS in Solids Testing by Automated Solvent Extraction Coupled with Reliable LC-MS/MS.**

**Ruth Marfil-Vega, Benedict Liu, Om Shrestha, Layla Abu-Al-Halaweh,  
Alicia Stell, Toshiya Matsubara**



# Recap from NEMC 2024

## EDGE PFAS

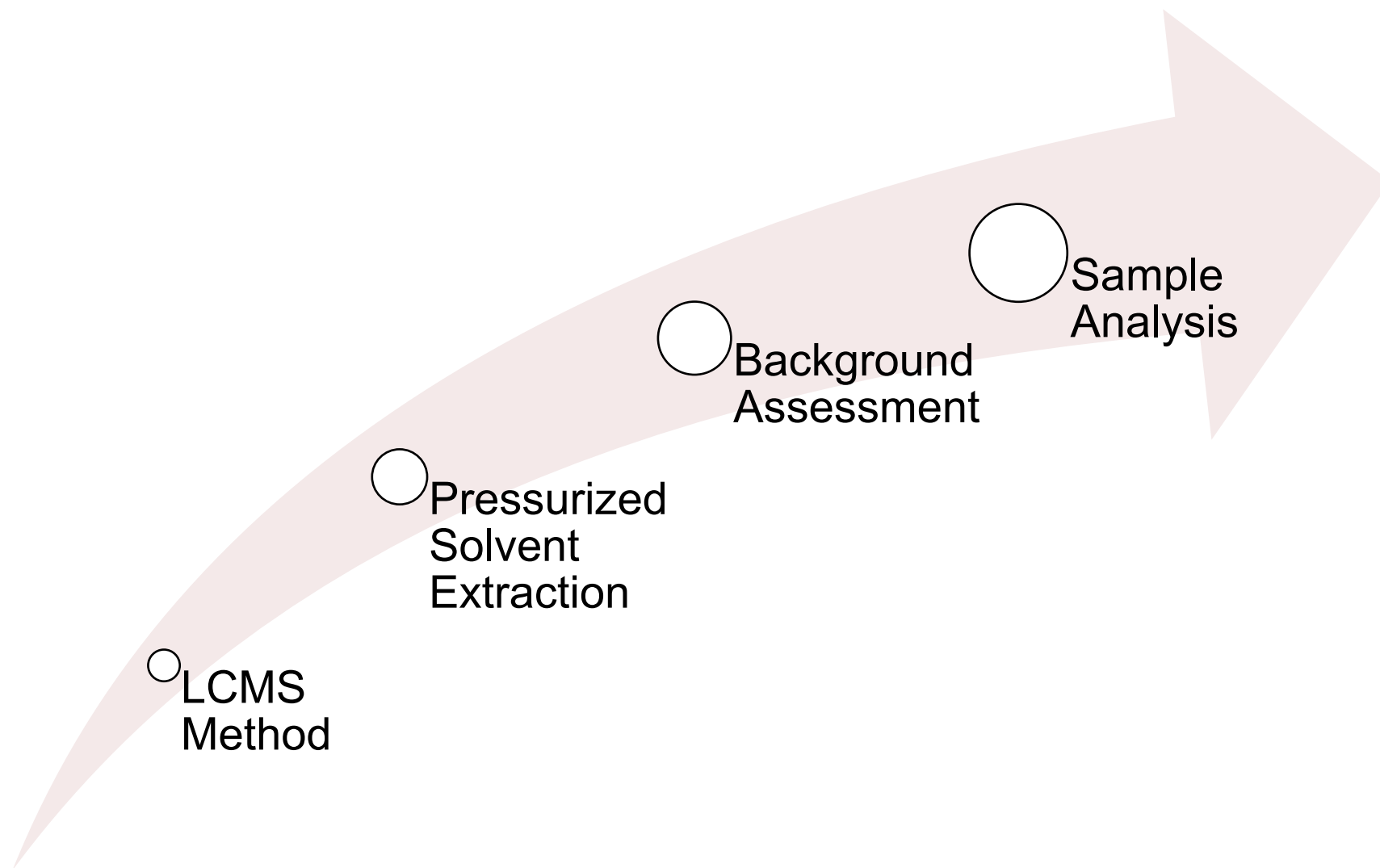


## What Happens When Automated Solvent Extraction and Robust LC-MS/MS are Coupled for Soil Analysis? By EPA 1633



| Parameter         | Value                                                      |
|-------------------|------------------------------------------------------------|
| Analytical Column | Shim-pack Scepter C18-120, 1.9 $\mu\text{m}$ , 2.0 x 50 mm |
| Delay Column      | Shim-pack Scepter C18-120, 3 $\mu\text{m}$ , 2.1 x 100 mm  |
| Injection Volume  | 5 $\mu\text{L}$                                            |
| Pretreatment Mode | Standard                                                   |
| Column Oven Temp. | 40°C                                                       |
| Mobile Phase      | A: 2 mM Ammonium Acetate in LCMS Grade Water               |
|                   | B: Acetonitrile                                            |
| Flow Rate         | 0.3 mL/min                                                 |
| Run Time          | 10 minutes                                                 |

# Recap from NEMC 2024

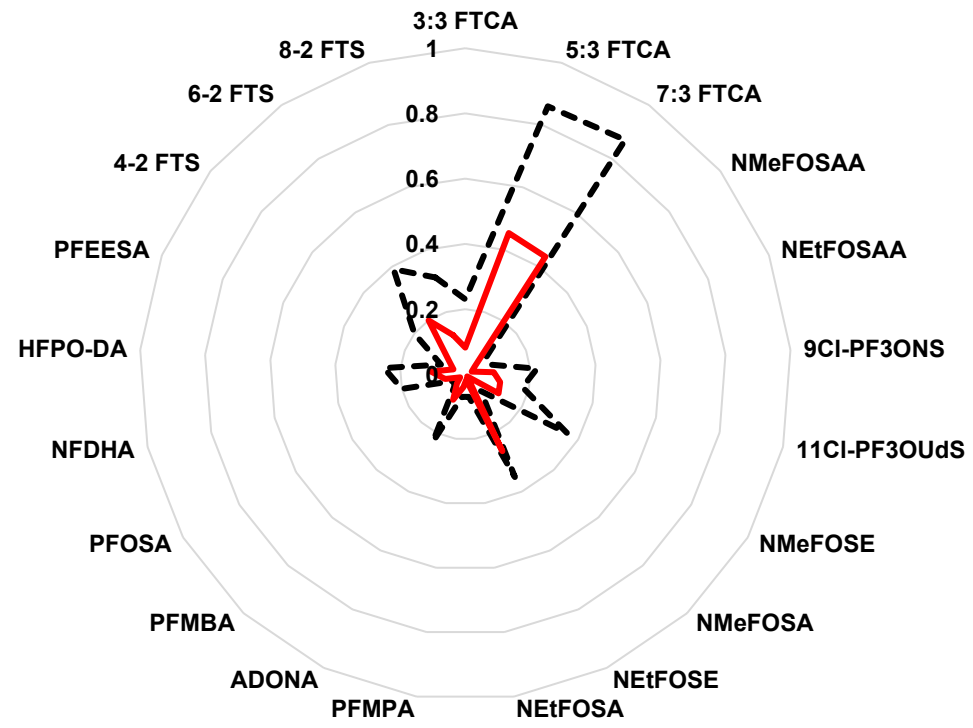
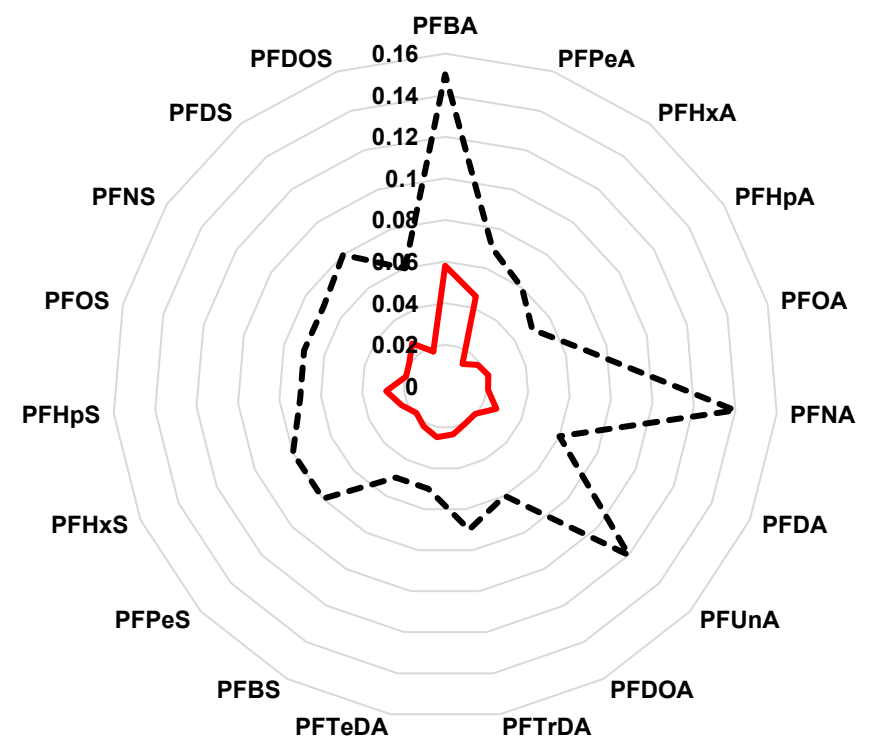


MDLs 2 times better than those reported in EPA Method 1633 in soils

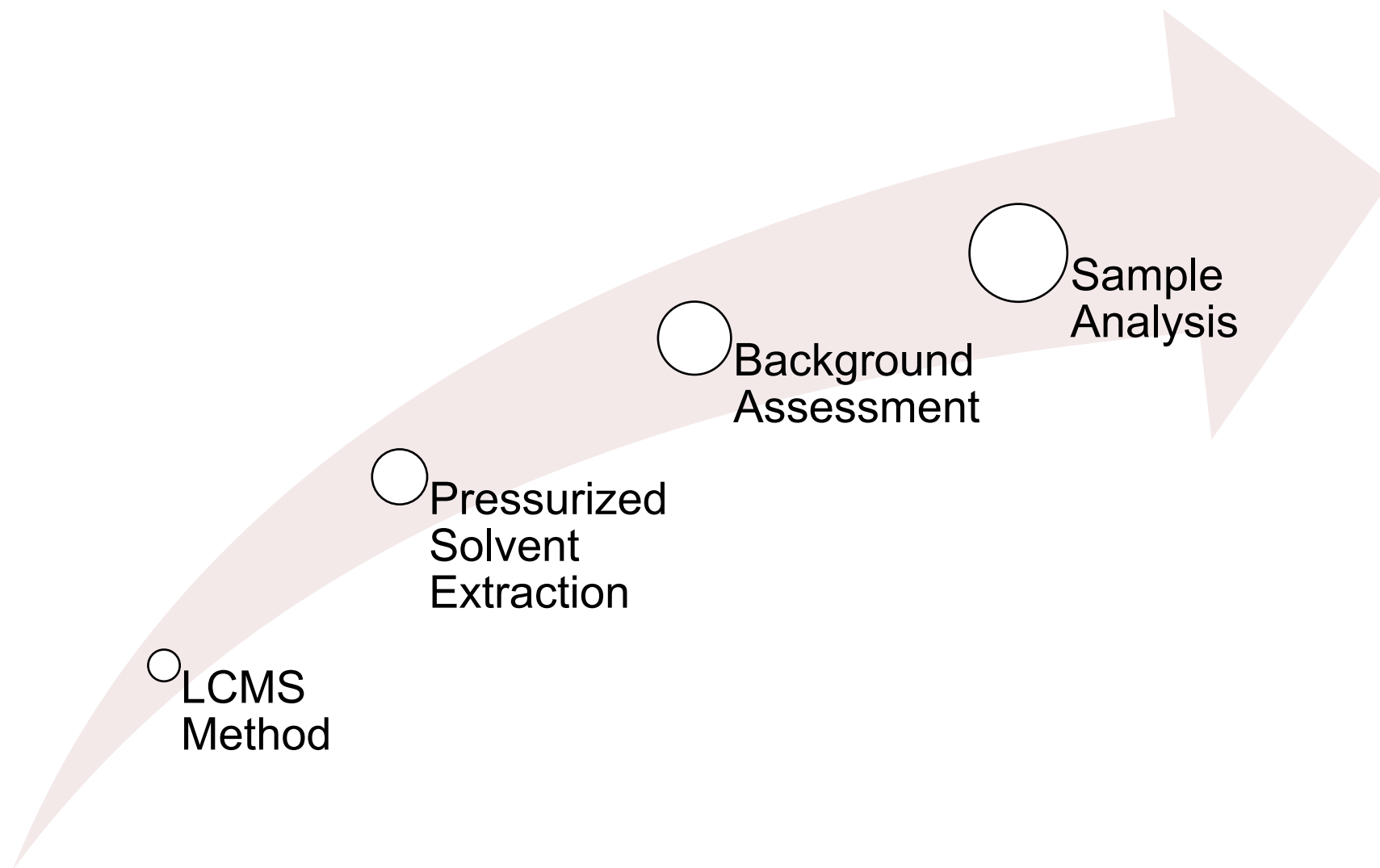
# Summary of MDLs (ng/g)

EPA reported MDLs

This work's MDLs



# Recap from NEMC 2024



MDLs 2 times better than those reported in EPA Method 1633 in soils



Demonstration done using Ottawa sand

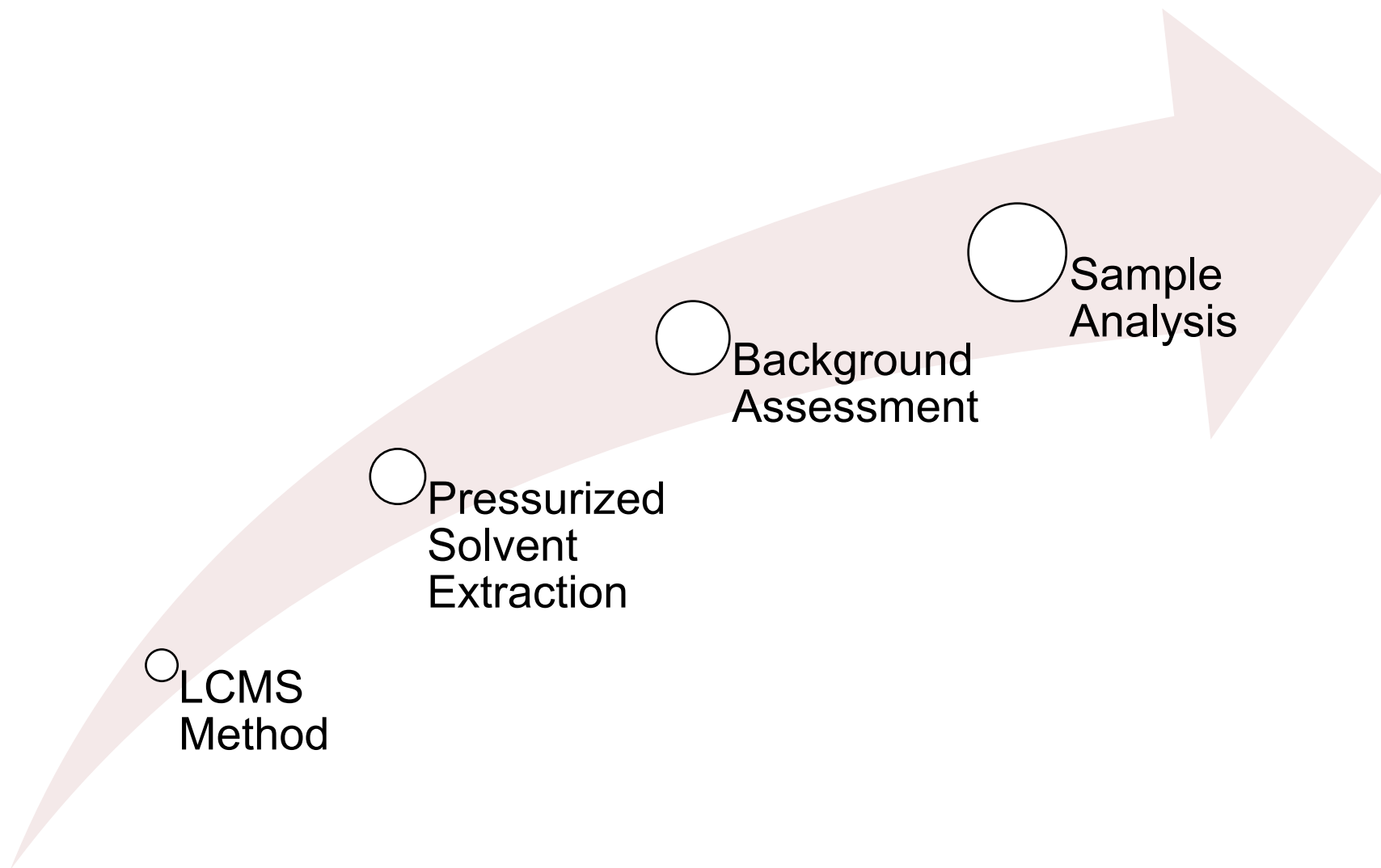
# From EPA 1633 to 1633A, and our new work



# Experimental Plan 2025

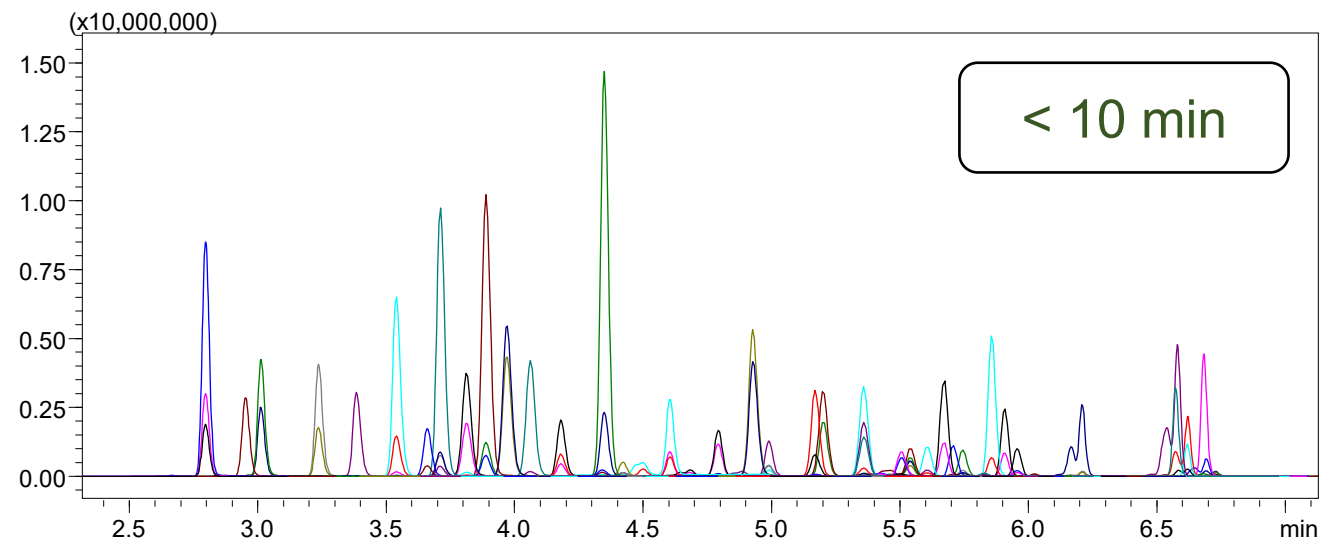
- Each sample type:
  - 2 g of tissue
    - a. Ground chicken
    - b. Ground fish
  - 5 g of soil
- Three spike concentrations tested:  
1/8 x EPA Cal 1, 1/4 x EPA Cal 1, 1/2 x EPA Cal 1
- Each concentration tested:  
Extraction batch:  
Spiked samples (n=7), unspiked samples (n=3), blank (n=1)  
Randomization of sample position in the extractor  
LCMS Batch analysis according to EPA 1633A

# Overall workflow

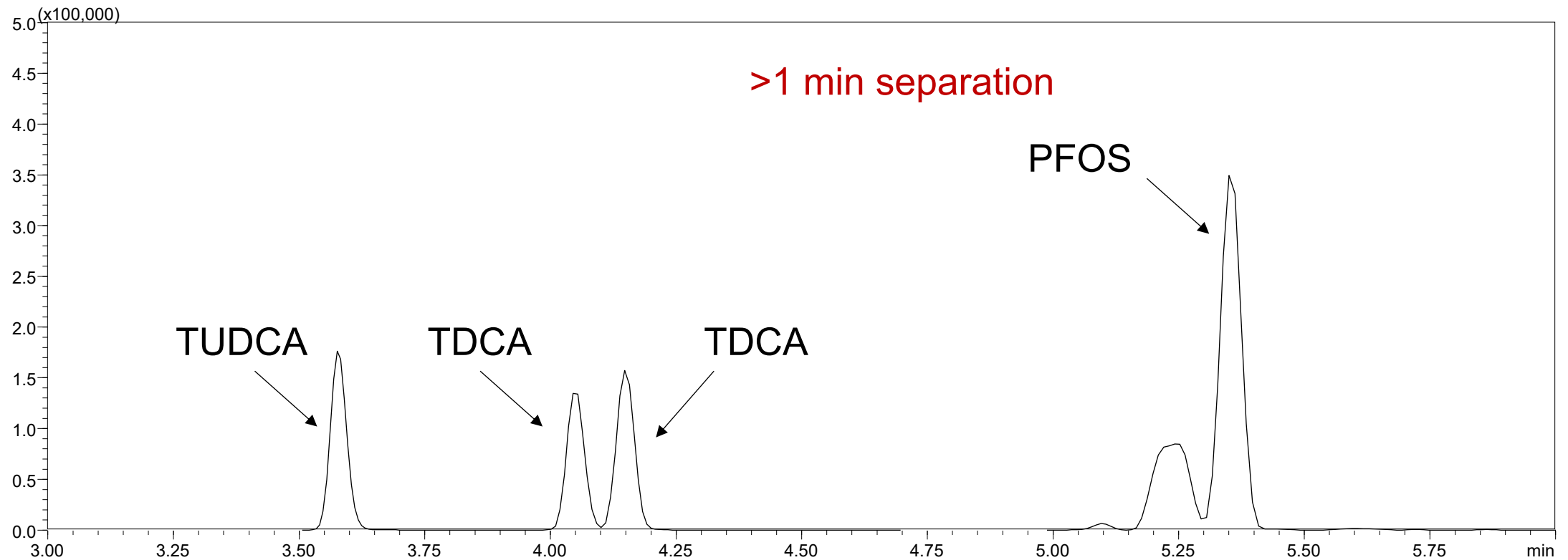


# LCMS Method

| Parameter         | Value                                                      |
|-------------------|------------------------------------------------------------|
| Analytical Column | Shim-pack Scepter C18-120, 1.9 $\mu\text{m}$ , 2.0 x 50 mm |
| Delay Column      | Shim-pack Scepter C18-120, 3 $\mu\text{m}$ , 2.1 x 100 mm  |
| Injection Volume  | 5 $\mu\text{L}$                                            |
| Pretreatment Mode | Standard                                                   |
| Column Oven Temp. | 40°C                                                       |
| Mobile Phase      | A: 2 mM Ammonium Acetate in LCMS Grade Water               |
|                   | B: Acetonitrile                                            |
| Flow Rate         | 0.3 mL/min                                                 |
| Run Time          | 10 minutes                                                 |



# Bile Salt Interference



# Calibration curve - Range

0.0025 – 2.5 ppb

|          |
|----------|
| PFHxA    |
| 5:3 FTCA |
| PFHpA    |
| PFPeS    |
| 6-2 FTS  |
| PFOA     |
| PFHxS    |
| PFNA     |
| 8-2 FTS  |
| NMeFOSAA |
| PFDA     |
| PFOS     |
| PFUnA    |
| PFNS     |
| PFDOA    |
| PFDS     |
| PFTTrDA  |
| PFTeDA   |
| PFOSA    |
| NMeFOSA  |
| NEtFOSA  |

0.005 – 5 ppb

|        |
|--------|
| PFMPA  |
| PFPeA  |
| PFMBA  |
| NFDHA  |
| PFEESA |

0.0125 – 2.5 ppb

|          |
|----------|
| PFHpS    |
| NEtFOSAA |
| PFDOS    |

0.0063 – 2.5 ppb

|      |
|------|
| PFBS |
|------|

0.01 - 10 ppb

|              |
|--------------|
| PFBA         |
| 4-2 FTS      |
| HFPO-DA      |
| ADONA        |
| 9Cl-PF3ONS   |
| 11Cl-PF3OUdS |

0.025 – 25 ppb

|          |
|----------|
| 7:3 FTCA |
| NMeFOSE  |
| NEtFOSE  |

0.0624 – 62.4 ppb

|          |
|----------|
| 3:3 FTCA |
|----------|

Cal 1

Cal 2

Cal 3

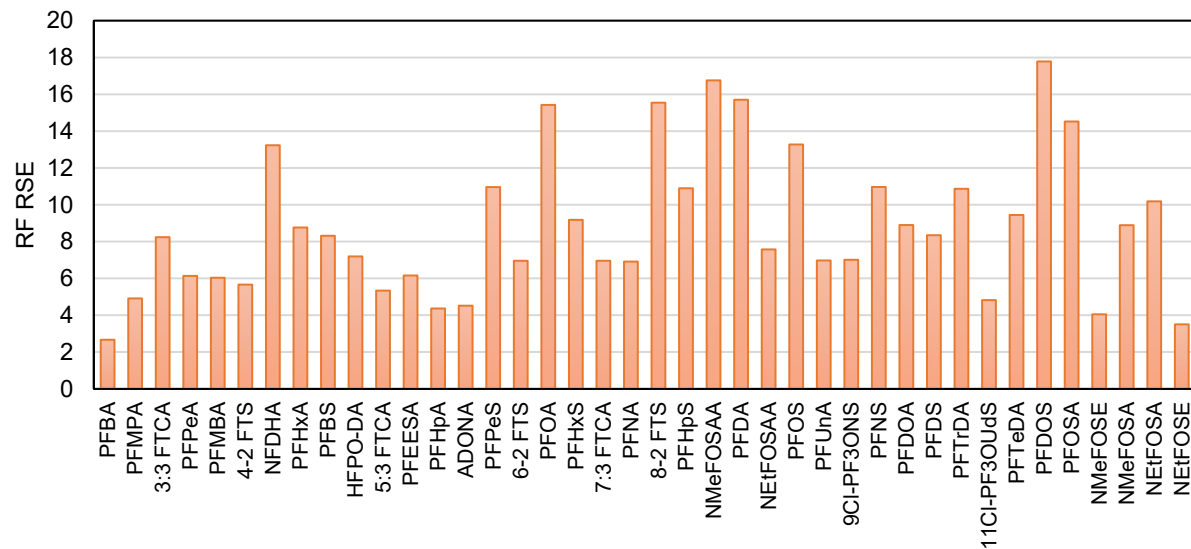
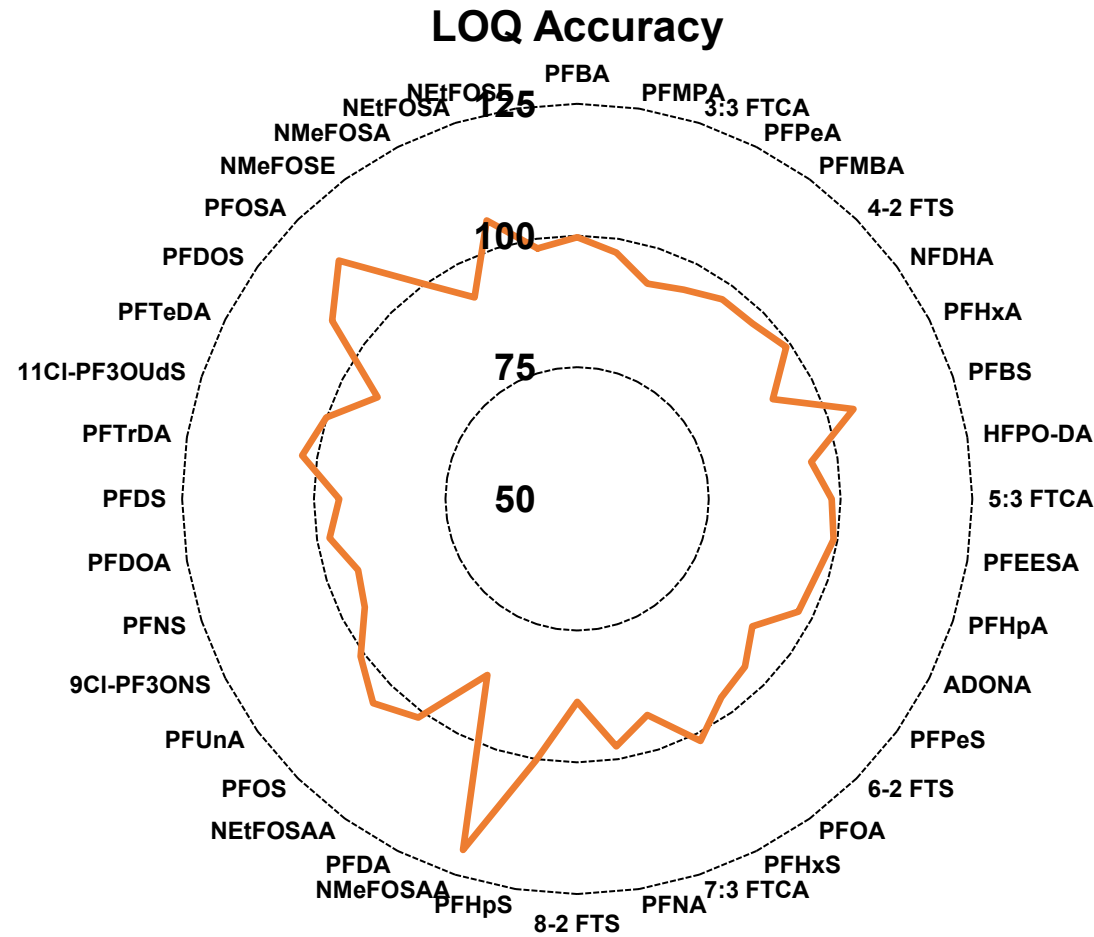
- Calibration curve range – variable, starting at **2.5 ppt in vial**
- Concentration of standards – lower than Cal 1 in EPA 1633A

Cal 1 = EPA Cal 1/80

Cal 2 = EPA Cal 1/40

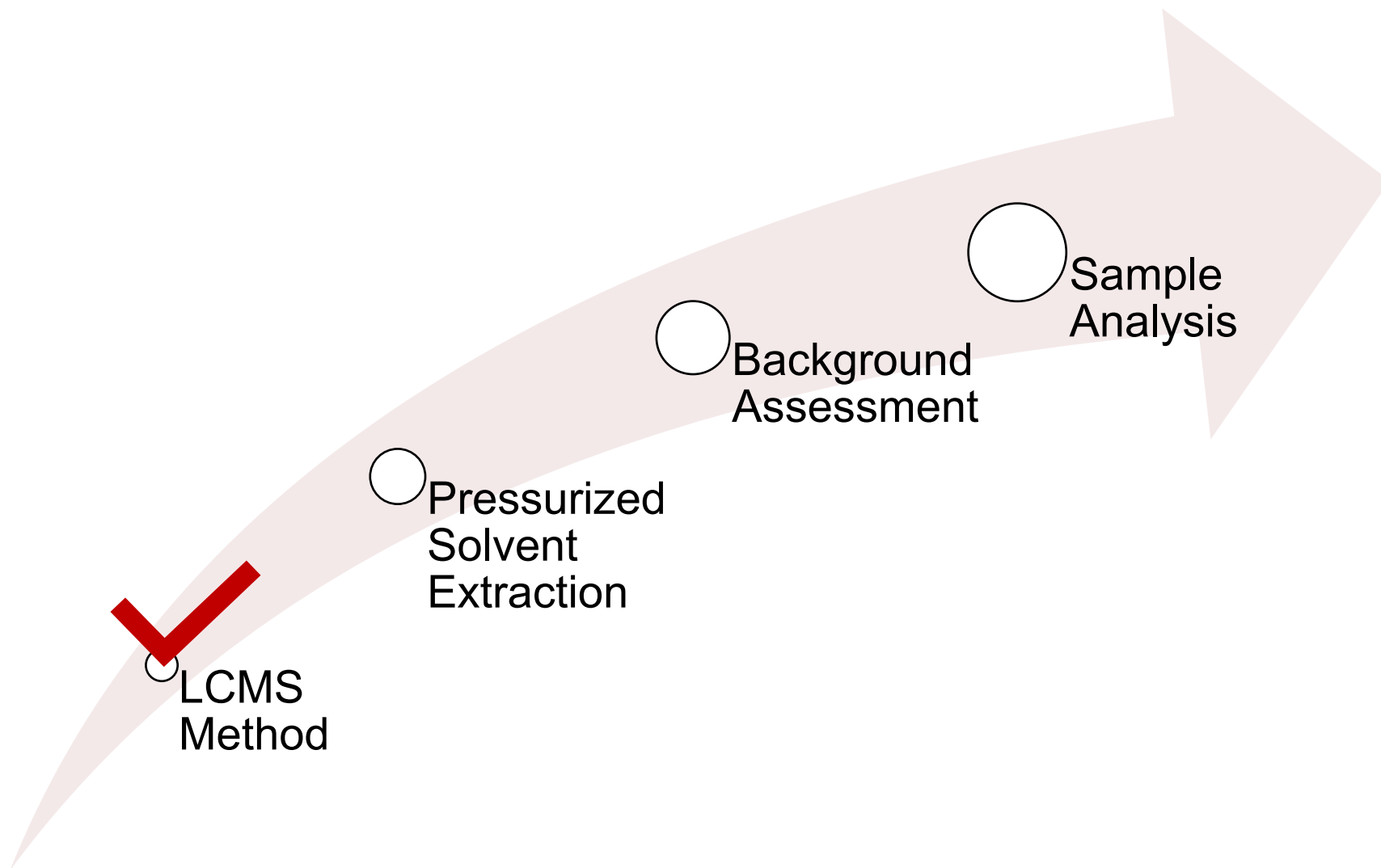
Cal 3 = EPA Cal 1/20

# Calibration Curve - Results



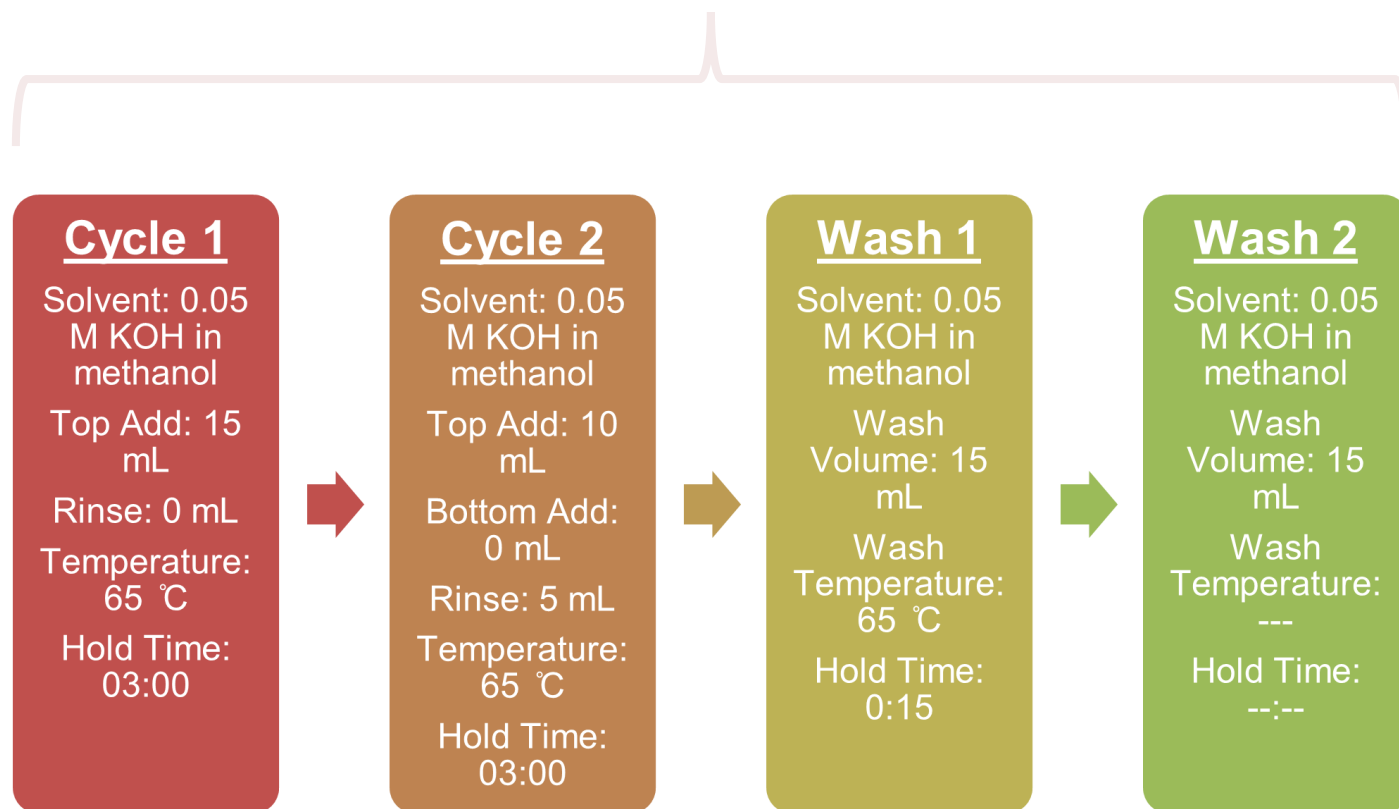
- %Accuracy LOQ and RF RSE within acceptable range from EPA 1633A

# Overall workflow



# Overall sample preparation 2024

## Extraction: EDGE PFAS



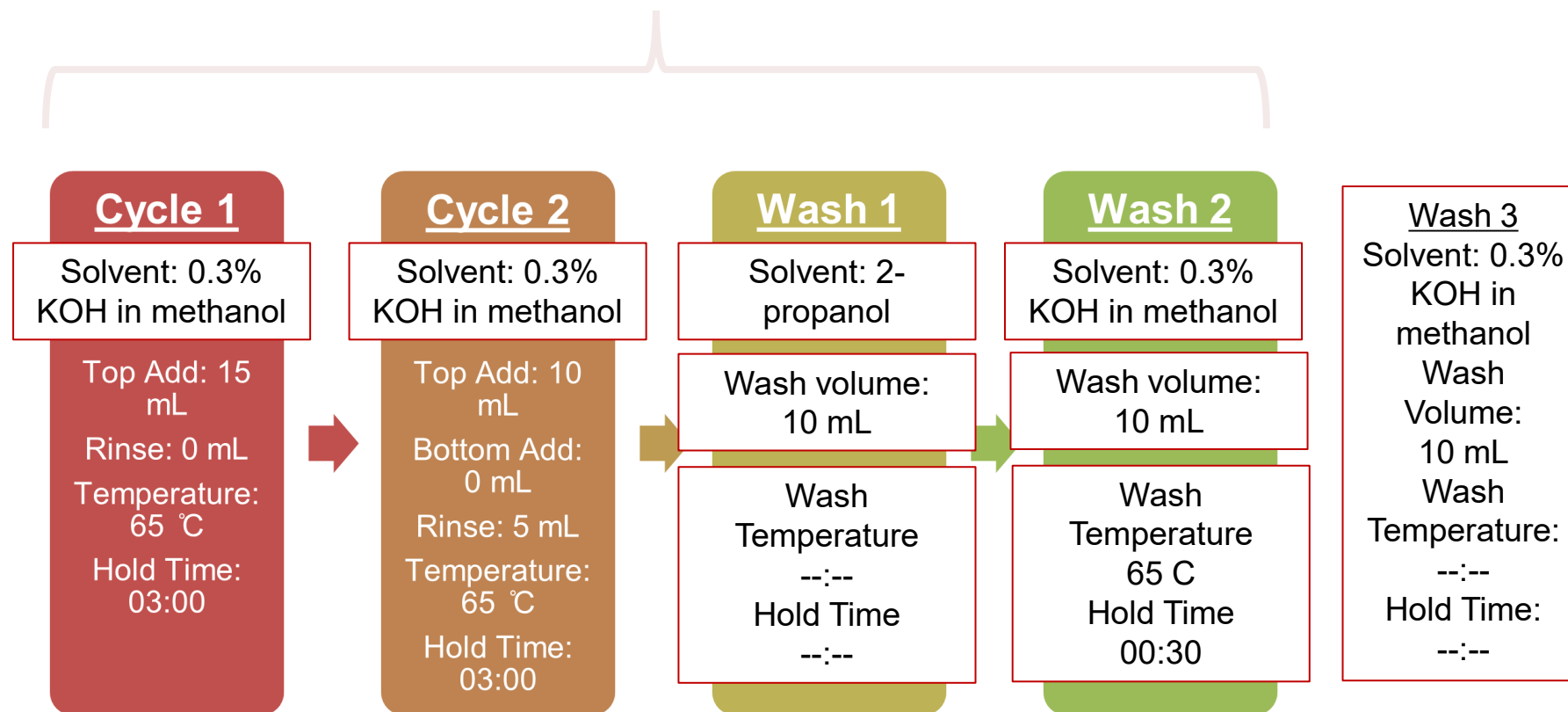
## Clean-up



- Carbon Black
- WAX SPE

# Overall sample preparation – Soil - 2025

## Extraction: EDGE PFAS

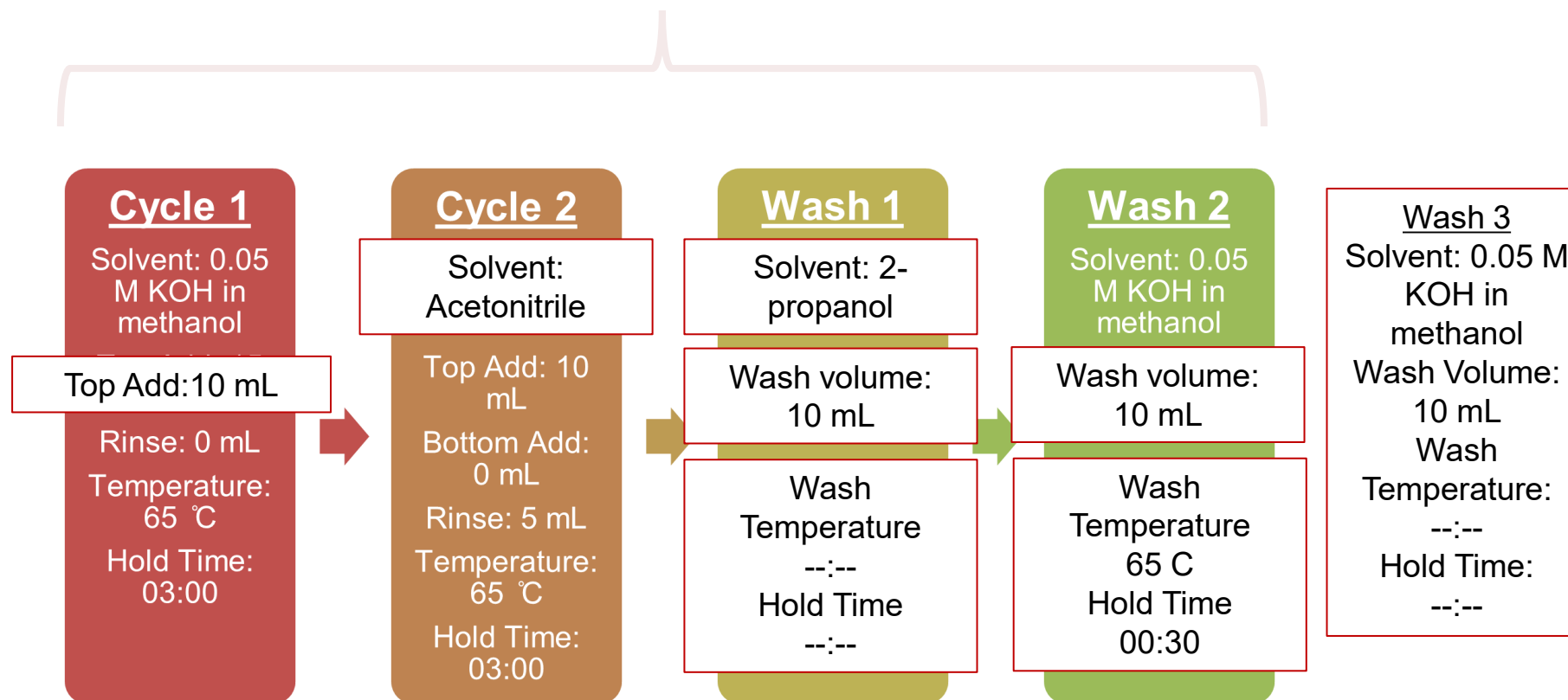


## Clean-up

- Carbon Black
- WAX SPE

# Overall sample preparation – Tissue - 2025

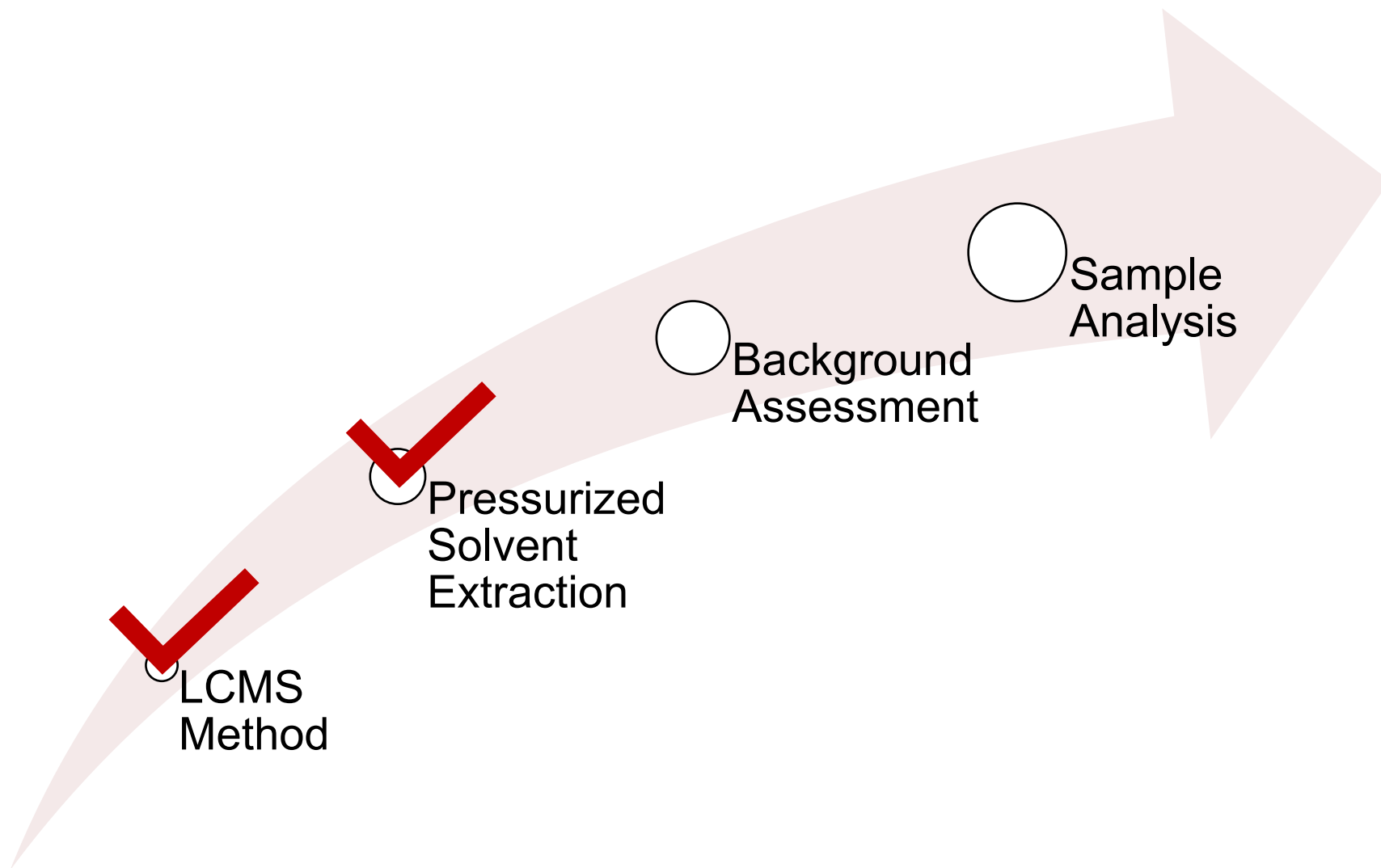
## Extraction: EDGE PFAS



## Clean-up

- Carbon Black
- WAX SPE

# Overall workflow



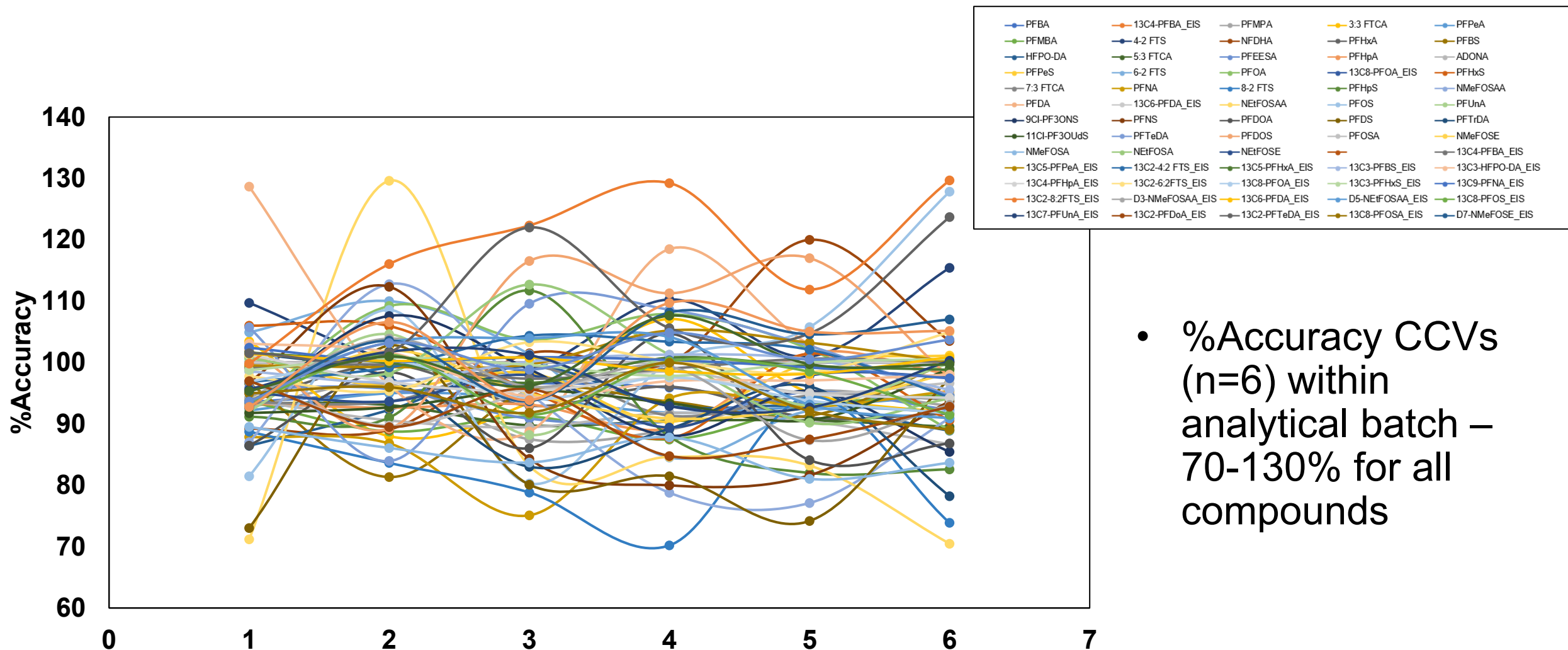
# Experimental Plan 2025

- Each sample type:
  - 2 g of tissue
    - a. Ground chicken
    - ~~b. Ground fish~~
  - ~~5 g of soil~~
- Three spike concentrations tested:
  - 1/8 x EPA Cal 1, 1/4 x EPA Cal 1, 1/2 x EPA Cal 1
- Each concentration tested:
  - Extraction batch:
  - Spiked samples (n=7), unspiked samples (n=3), blank (n=1)
  - Randomization of sample position in the extractor
  - LCMS Batch analysis according to EPA 1633A



Lost samples during shipping

# LCMS Batch Analysis - CCVs



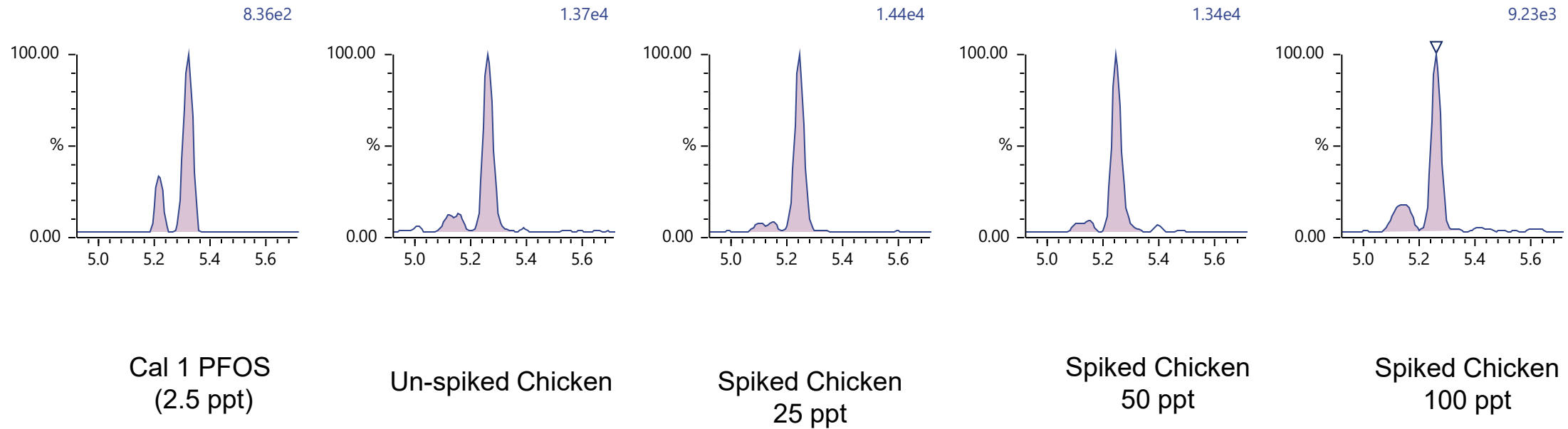
- %Accuracy CCVs (n=6) within analytical batch – 70-130% for all compounds

# Method Detection Limits - Tissue

- Chicken samples
  - Unspiked – only compound quantified PFOS
- Spiked samples -1/8 x EPA Cal 1, 1/4 x EPA Cal 1, 1/2 x EPA Cal 1

|             | EPA              | 1/2x                    | 1/4x                 | 1/8x                 |
|-------------|------------------|-------------------------|----------------------|----------------------|
| Highest MDL | 2.38<br>5:3 FTCA | 1.772<br><i>NEtFOSE</i> | 0.625<br><i>PFOS</i> | 1.070<br><i>PFOS</i> |
| Lowest MDL  | 0.069<br>PFOSA   | 0.041<br>8-2 FTS        | 0.010<br>8-2 FTS     | 0.003<br>8-2 FTS     |

# PFOS Background



Cal 1 PFOS  
(2.5 ppt)

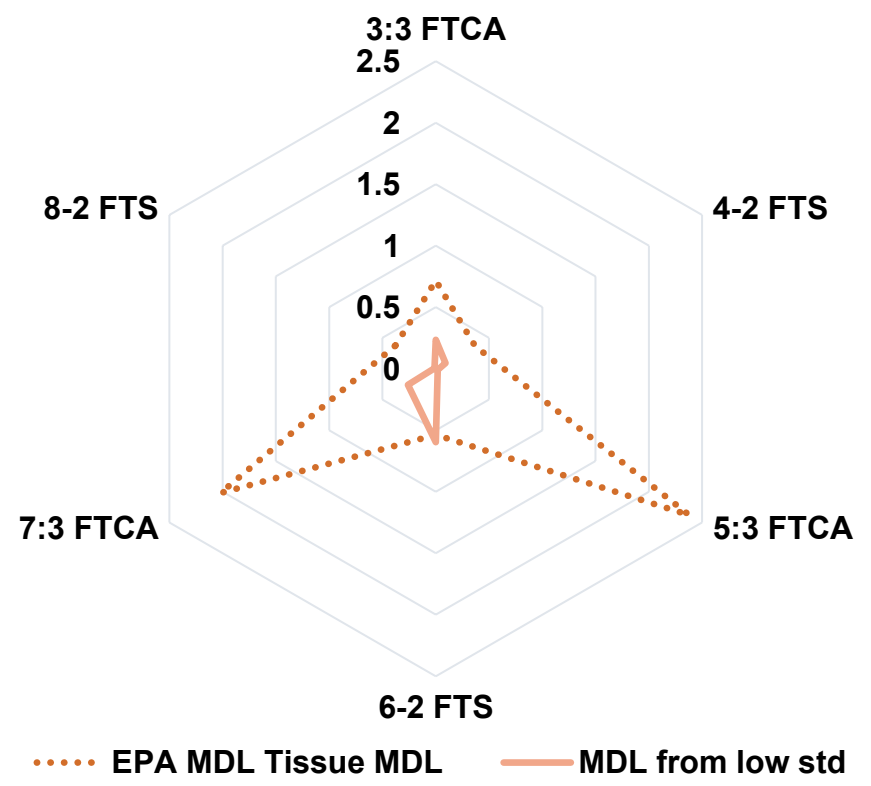
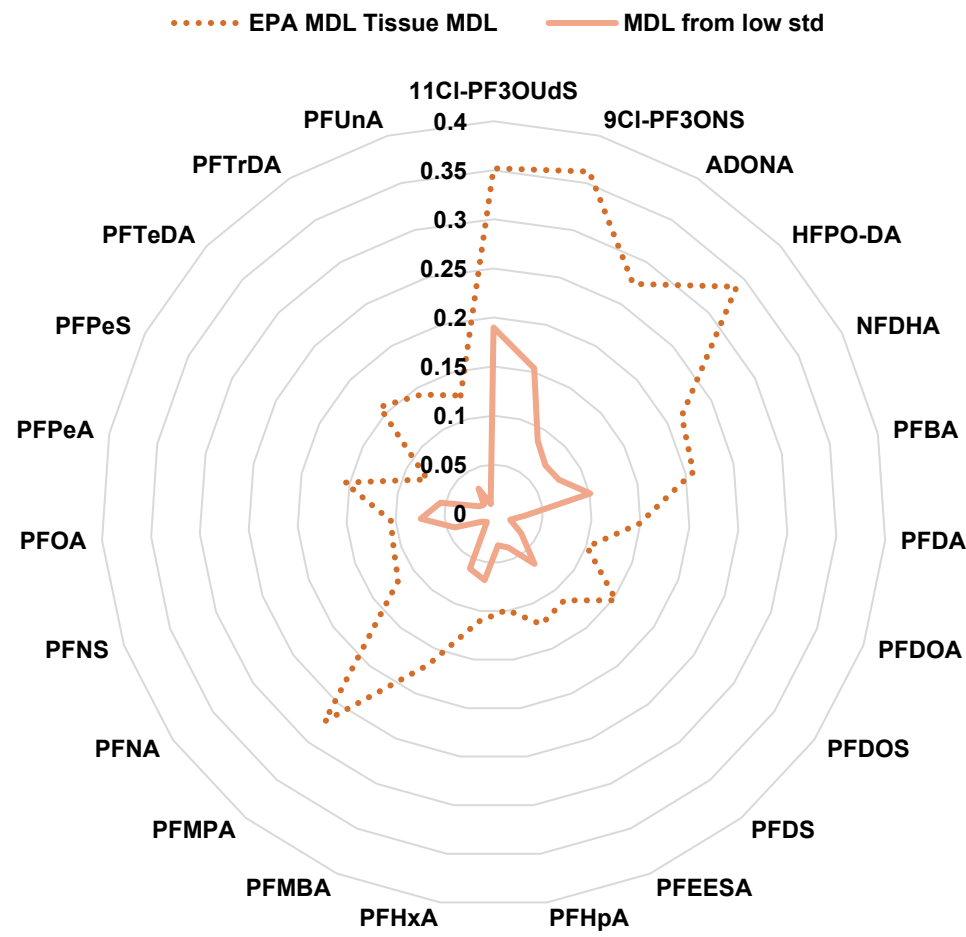
Un-spiked Chicken

Spiked Chicken  
25 ppt

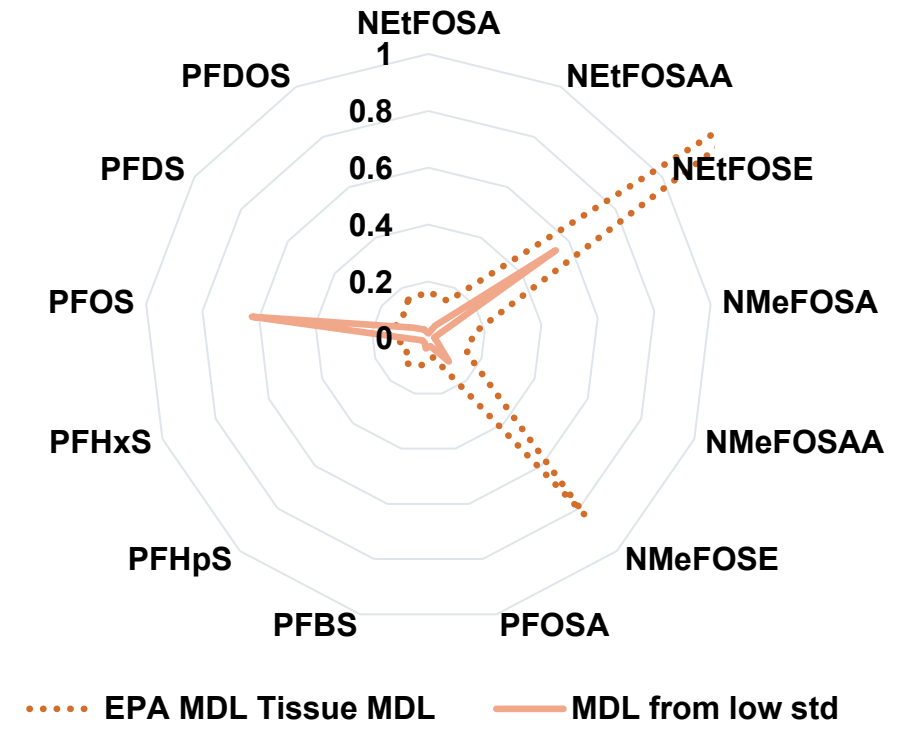
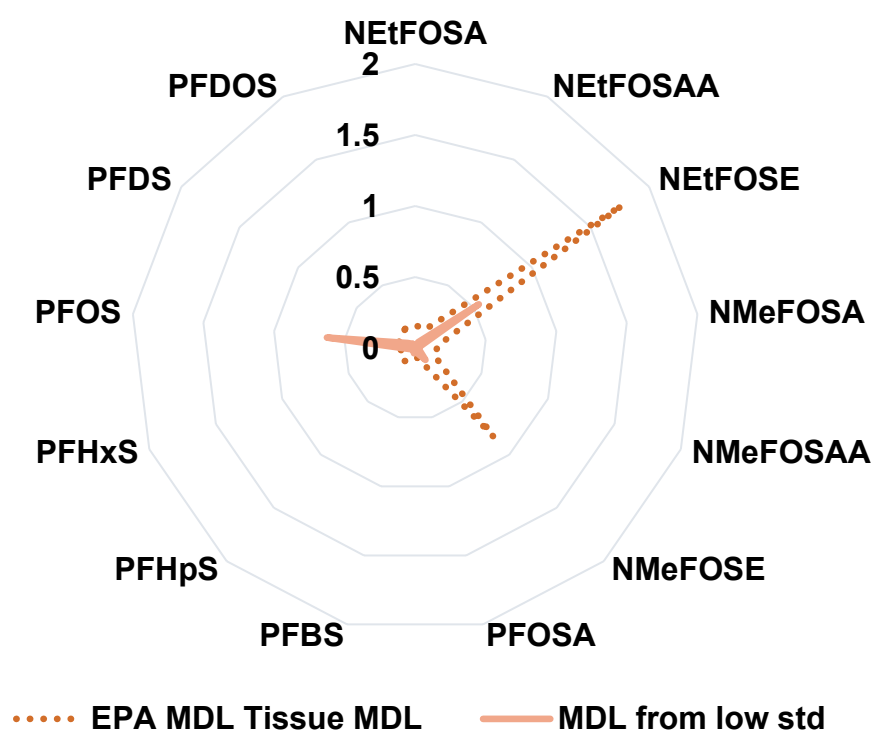
Spiked Chicken  
50 ppt

Spiked Chicken  
100 ppt

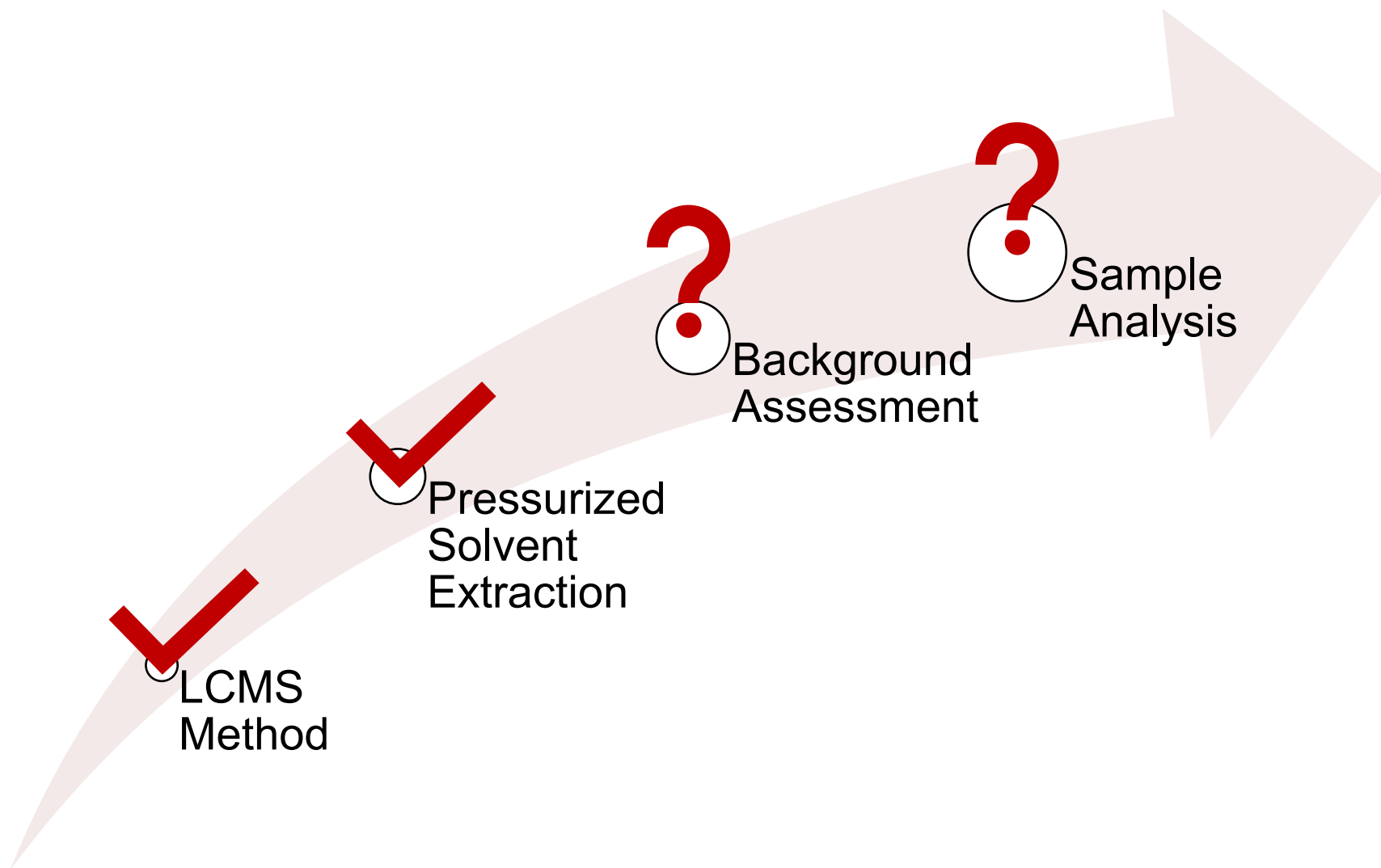
# Method Detection Limits - Tissue



# Method Detection Limits - Tissue



# Overall workflow



# Conclusions



- Performance of updated LCMS method demonstrated
  - Sensitivity
  - Robustness
- On-going demonstration of performance of the overall workflow for tissue and soils samples

# Acknowledgements

**Om Shrestha, SSI**



**Benedict Liu, CEM**



**Toshiya  
Matsubara, SSI**



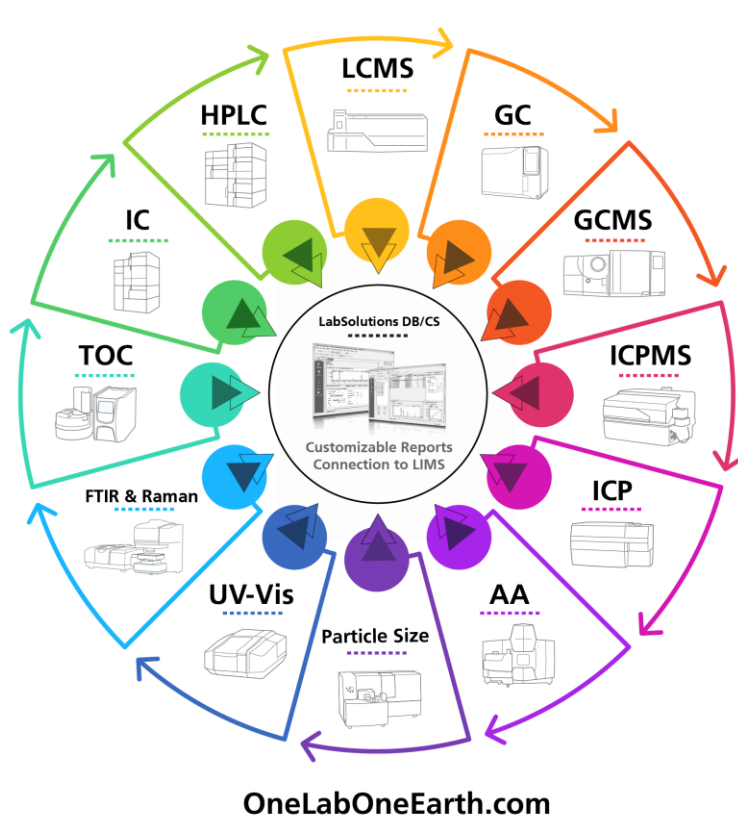
**Alicia Stell, CEM**



**Eishi Imoto, SSI**

**Layla Abu-Al-Halaweh, CEM**

|               |                                           |
|---------------|-------------------------------------------|
| HPLC          | Carbamate<br>Diquat<br>Glyphosate         |
| IC            | Anions<br>Chromium VI<br>Ammonia          |
| TOC           | Nitrogen<br>Organic Carbon<br>Phosphorous |
| FTIR & Raman  | <b>Microplastics</b>                      |
| UV-Vis        | Chlorine<br>UV254<br>Others               |
| Particle Size | <b>Solids</b>                             |



|                                                                                    |       |
|------------------------------------------------------------------------------------|-------|
| Cyanotoxins<br>PFAS<br>Pesticides<br>Emerging Contaminants<br>Unknowns (QTOF)      | LCMS  |
| HAAs<br>Herbicides<br>Pesticides                                                   | GC    |
| Phenols<br>Other Organics<br>PCBs                                                  | GC    |
| 1,4-dioxane<br>Dioxins & Furans<br>Nitrosamines<br>Other Organics<br>Microplastics | GCMS  |
| Semivolatiles<br>Taste & Odor<br>Volatiles<br>THMs                                 | GCMS  |
| <b>Metals</b>                                                                      | ICPMS |
| <b>Metals</b>                                                                      | ICP   |
| <b>Metals</b>                                                                      | AA    |

For any questions, contact:  
**Ruth Marfil-Vega**  
[rmmarfilvega@shimadzu.com](mailto:rmmarfilvega@shimadzu.com)

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