Considerations when analysing PFAS containing samples

Matt James, Tony Edge

avantor™

Agenda

01

- Introduction to PFAS

02

- Contamination issues with PFAS

03

- Use of delay columns

04

- Sample preparation techniques for environmental analysis

05

- Conclusions

06

Introduction to PFAS

- Per- and polyfluoroalkyl substances are a group of synthetic organofluorine chemical compounds that have multiple fluorine atoms attached to an alkyl chain
- Often referred to as forever chemicals due to their longevity in the environment, they have also been found in a wide range of marine life
- US EPA toxicity database, DSSTox, lists 14,735 unique PFAS chemical compounds,[7] while PubChem lists approximately 6 million
- Many PFASs were used for their enhanced water-resistant properties
- Residues have been detected in humans and wildlife, prompting concern about impacts to health.[9][10][11] According to the National Academies of Sciences, Engineering, and Medicine
- PFAS exposure is linked to increased risk of dyslipidemia (abnormally high cholesterol), suboptimal antibody response, reduced infant and fetal growth, and higher rates of kidney cancer.[12]

Challenges to developing PFAS workflow

- PFAS are ubiquitous within the laboratory environment
  - Very challenging to prevent environmental contamination
  - Use of delay columns reduces potential interferences derived from analytical system
- Compounds are thought to be highly toxic and so detection limits are very low
  - Typically 1 ng/L
  - Often requires pre-concentration using SPE, e.g. factor of 250x according to EPA 537.1
  - Low detection limits require LC-MS/MS
- Some PFAS compounds will adsorb to plastic and glass surfaces, reducing recovery
  - PFAS compounds can also stick to Aluminium
- Toxicity concerns mean that extra handling precautions should be taken
  - This can be a source of possible contamination

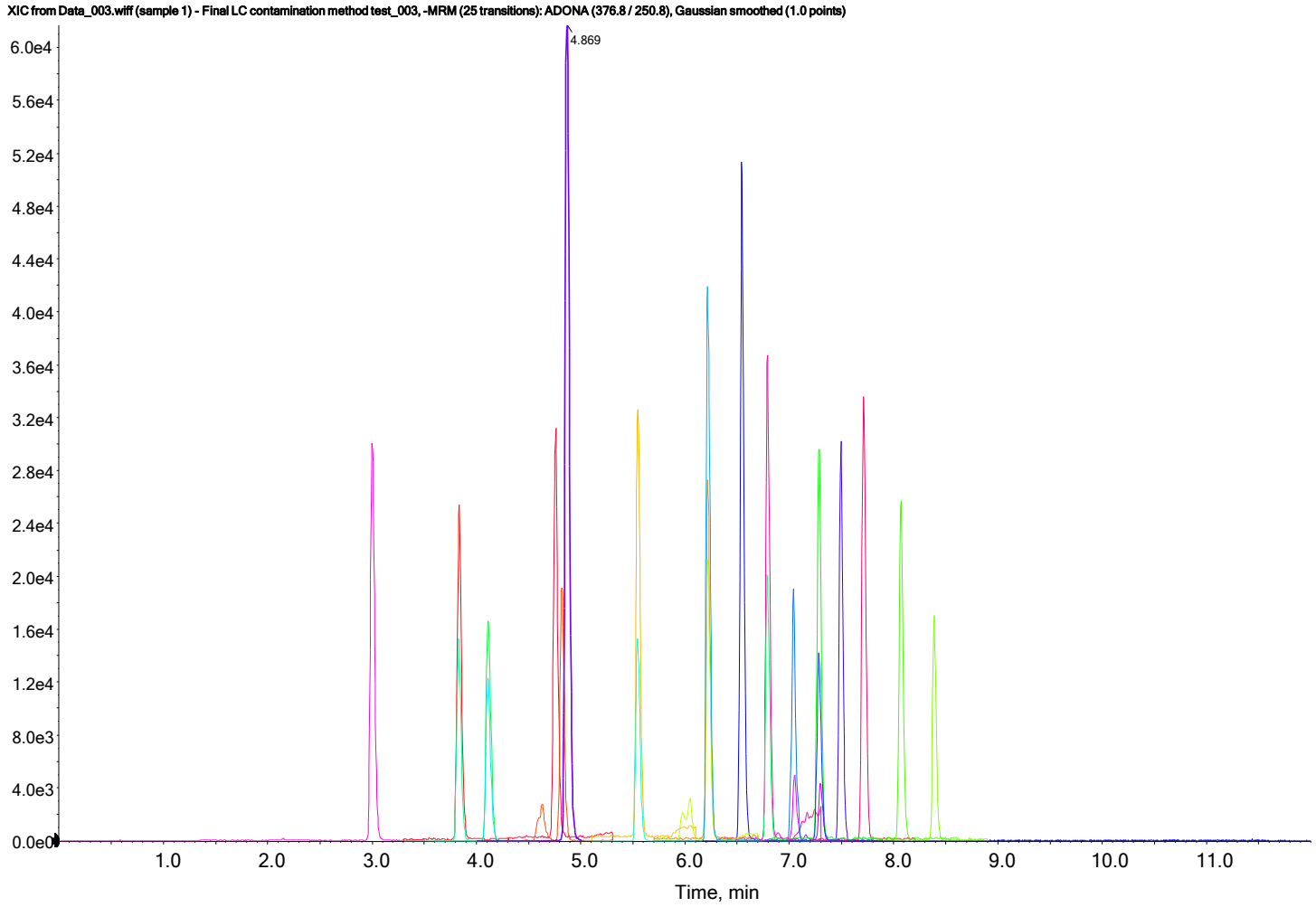
List of EPA 537.1 compounds and MRLs

TABLE 5. DLs AND LCMRLs IN REAGENT WATER

Analyte	Fortified Conc. (ng/L)[a]	DL[b] (ng/L)	LCMRL[c] (ng/L)
PFBS	4.0	1.8	6.3
PFHxA	4.0	1.0	1.7
HFPO-DA	4.0	1.9	4.3
PFHpA	4.0	0.71	0.63
PFHxS	4.0	1.4	2.4
ADONA	4.0	0.88	0.55
PFOA	4.0	0.53	0.82
PFOS	4.0	1.1	2.7
PFNA	4.0	0.70	0.83
9Cl-PF3ONS	4.0	1.4	1.8
PFDA	4.0	1.6	3.3
NMeFOSAA	4.0	2.4	4.3
PFUnA	4.0	1.6	5.2
NEtFOSAA	4.0	2.8	4.8
11CL-PF3OUdS	4.0	1.5	1.5
PFDoA	4.0	1.2	1.3
PFTrDA	4.0	0.72	0.53
PFTA	4.0	1.1	1.2

PFAS Method: contamination testing

- EPA 537.1 method – This method is used for contamination testing

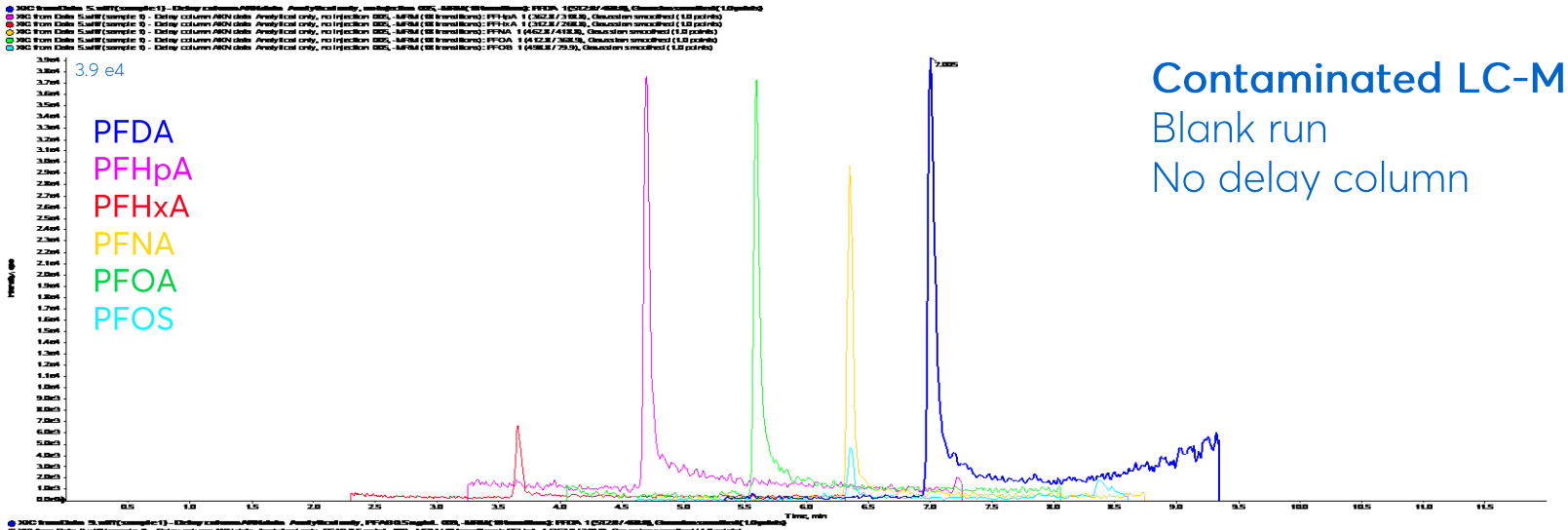


Column:	Avantor® ACE® UltraCore SuperC18, 2.5 µm, 100 x 2.1 mm
Delay column:	Avantor® ACE® PFAS Delay Column, 50 x 2.1 mm
Mobile Phases:	A: 20 mM ammonium acetate B: MeOH

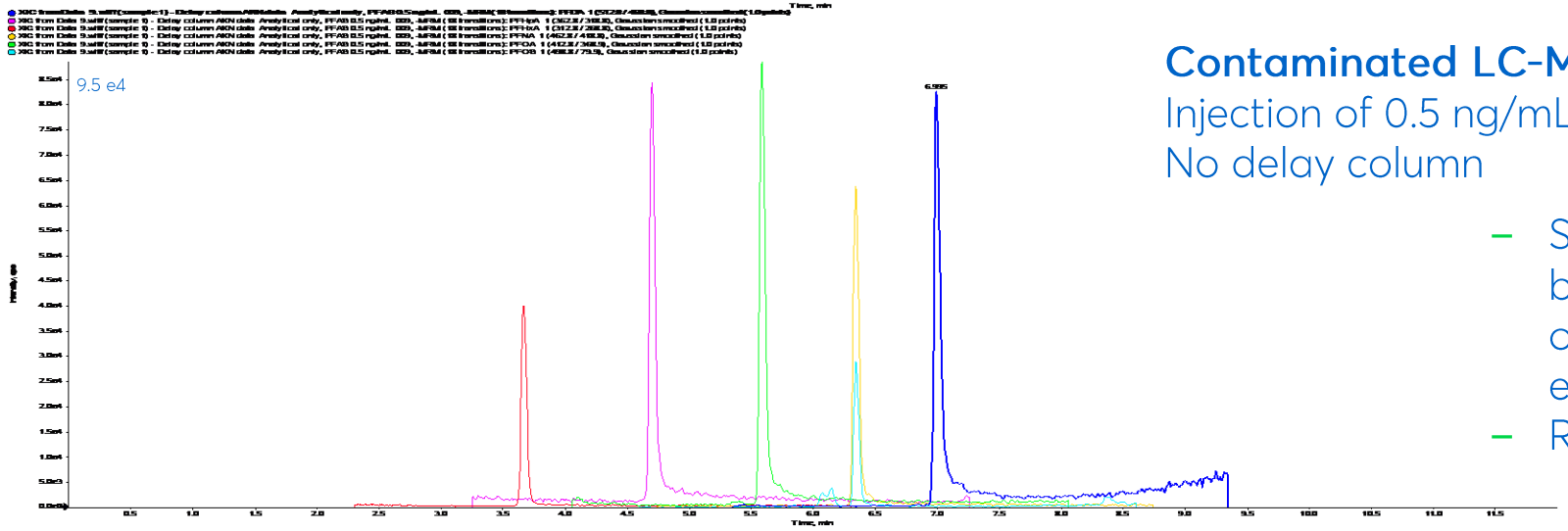
Time (mins)	% B
0	5
0.1	40
8.5	95
10.5	95
10.6	5
13.2	5

Flow Rate:	0.4 mL/min
Temperature:	40 °C
Detection:	Sciex QTRAP® 6500+ LC-MS/MS Ionisation mode: ESI, negative mode; Source temperature: 450 °C; Curtain gas: 30 psig; Ionspray™ source voltage: -4500 V
Sample:	EPA 537.1 mix (18 analytes), 3 internal standards and 4 surrogates

PFAS Method: Delay column use



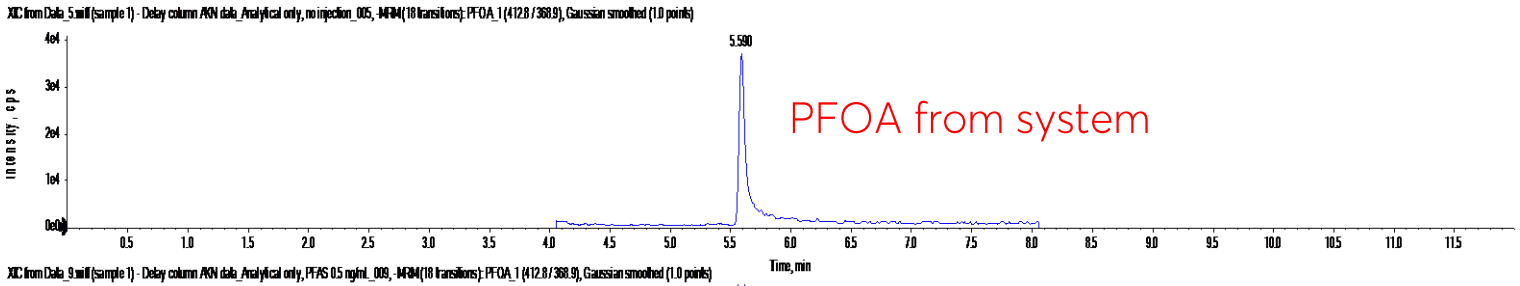
Contaminated LC-MS system
Blank run
No delay column



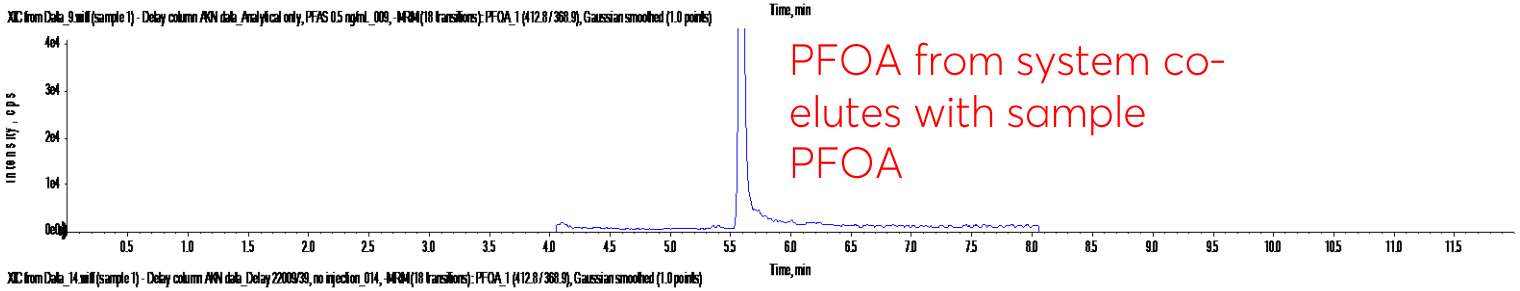
Contaminated LC-MS system
Injection of 0.5 ng/mL PFAS standards
No delay column

- Sample PFAS coelute with system background PFAS that accumulate on–column during post gradient re-equilibration
- Results in inaccurate quantitation

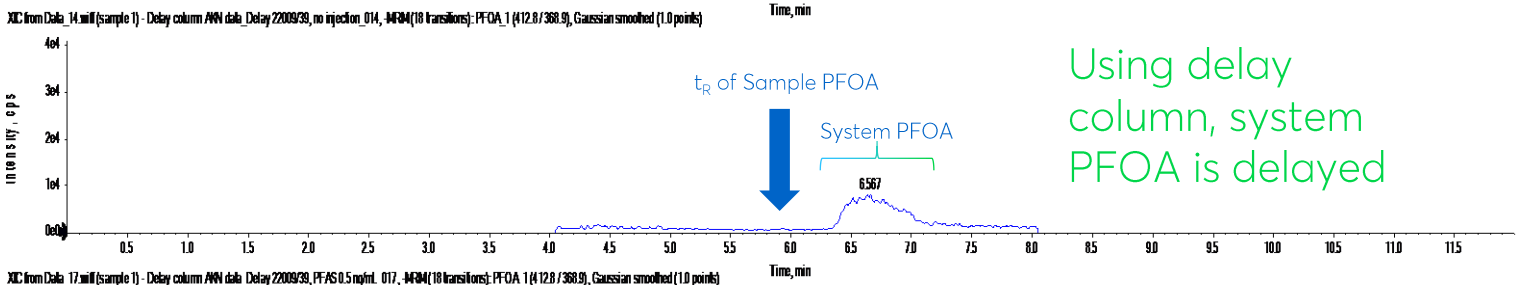
PFAS Method: Delay column use - PFOA



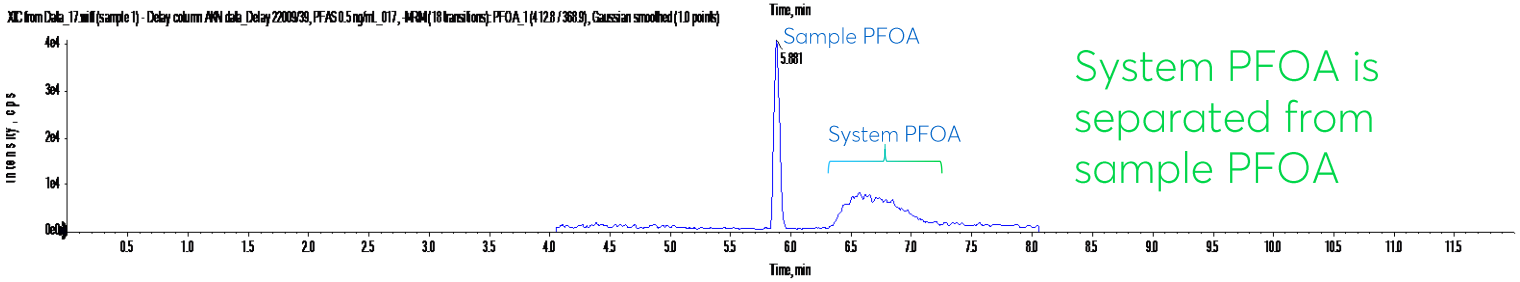
PFOA from system



PFOA from system co-elutes with sample PFOA



Using delay column, system PFOA is delayed



System PFOA is separated from sample PFOA

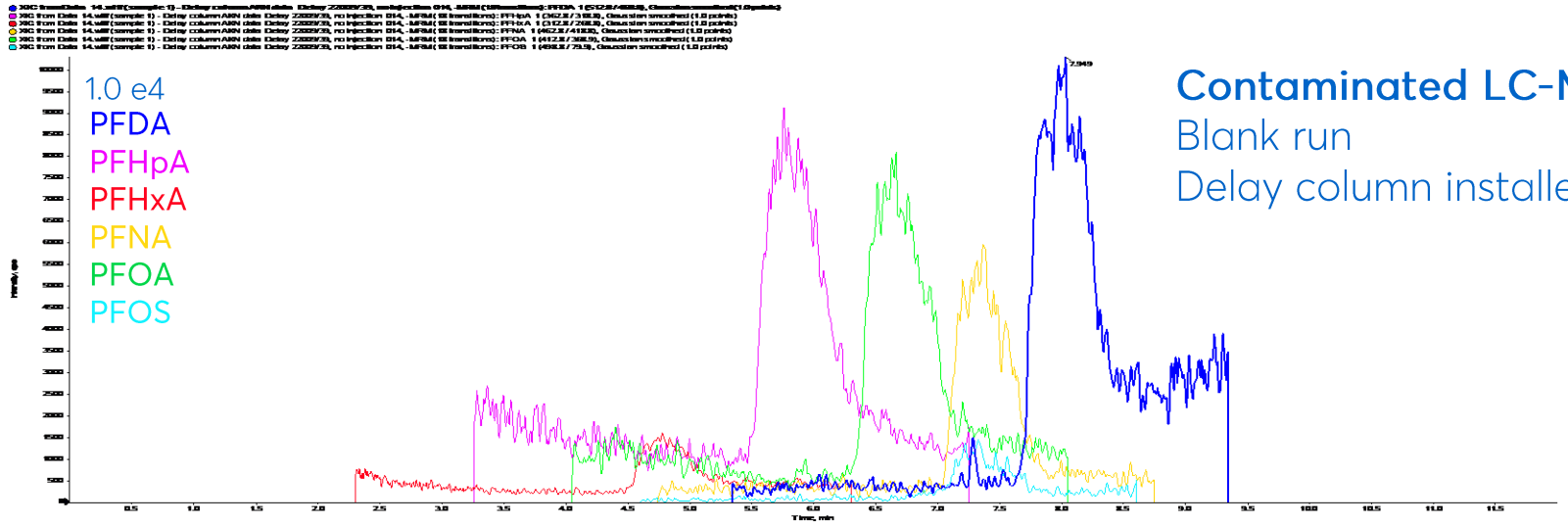
Contaminated LC-MS system
Blank run
No delay column

Contaminated LC-MS system
Injection of 0.5 ng/mL PFAS standards
No delay column

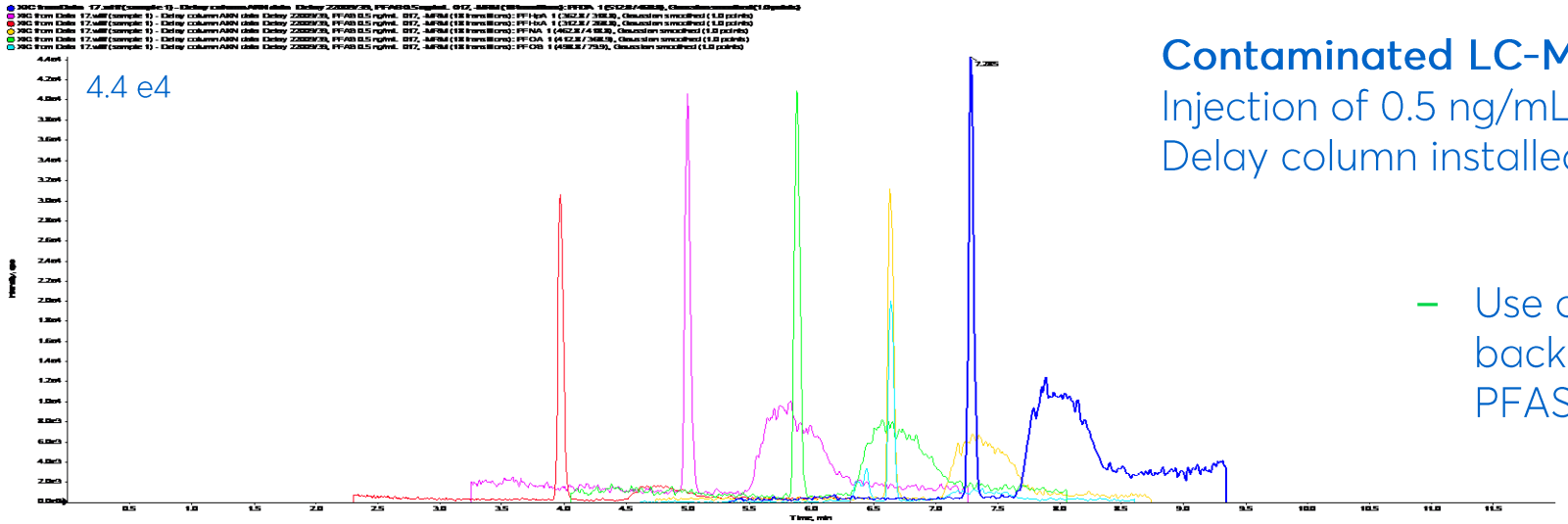
Contaminated LC-MS system
Blank run
Delay column installed

Contaminated LC-MS system
Injection of 0.5 ng/mL PFAS standards
Delay column installed

PFAS Method: Delay column use



Contaminated LC-MS system
Blank run
Delay column installed

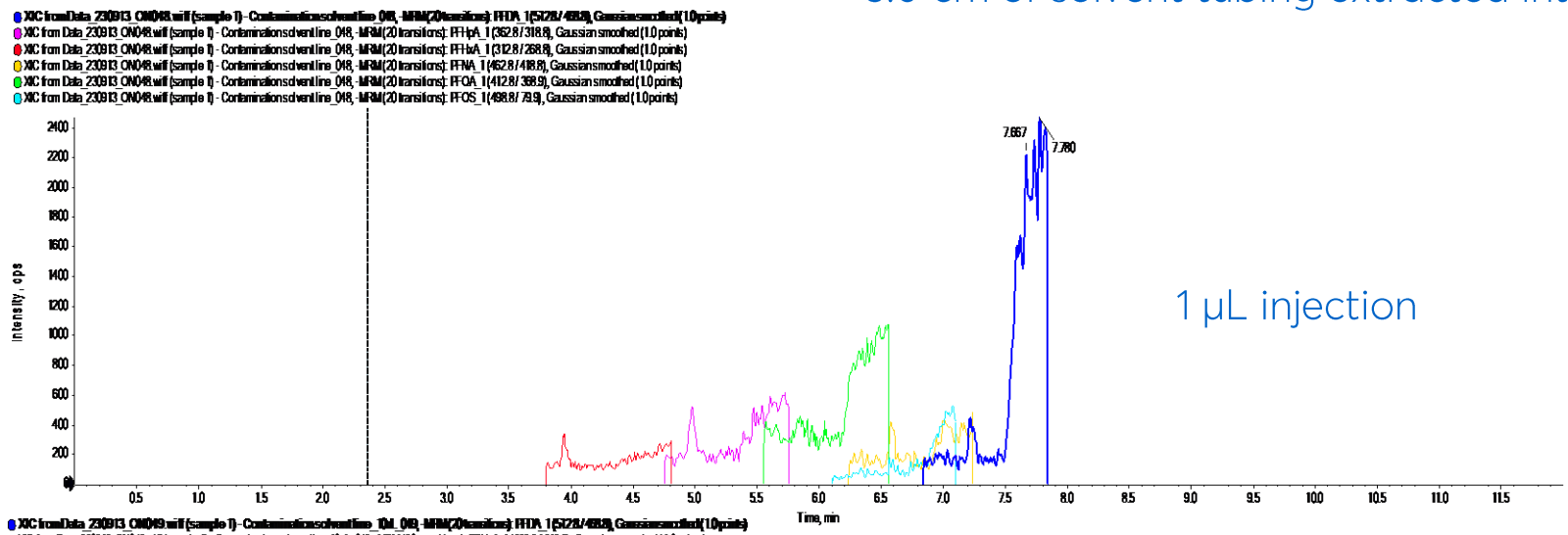


Contaminated LC-MS system
Injection of 0.5 ng/mL PFAS standards
Delay column installed

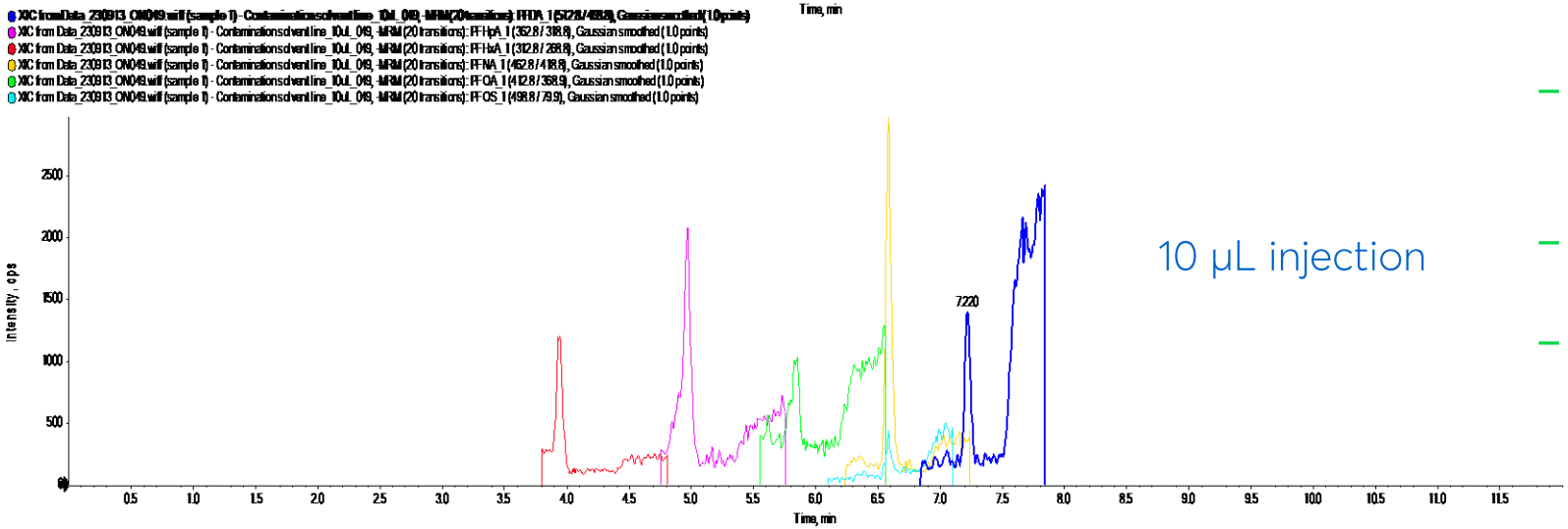
- Use of a delay column retains system background PFAS until after sample PFAS have eluted

Tracking system contamination: solvent tubing

3.0 cm of solvent tubing extracted into 1.5 mL MeOH



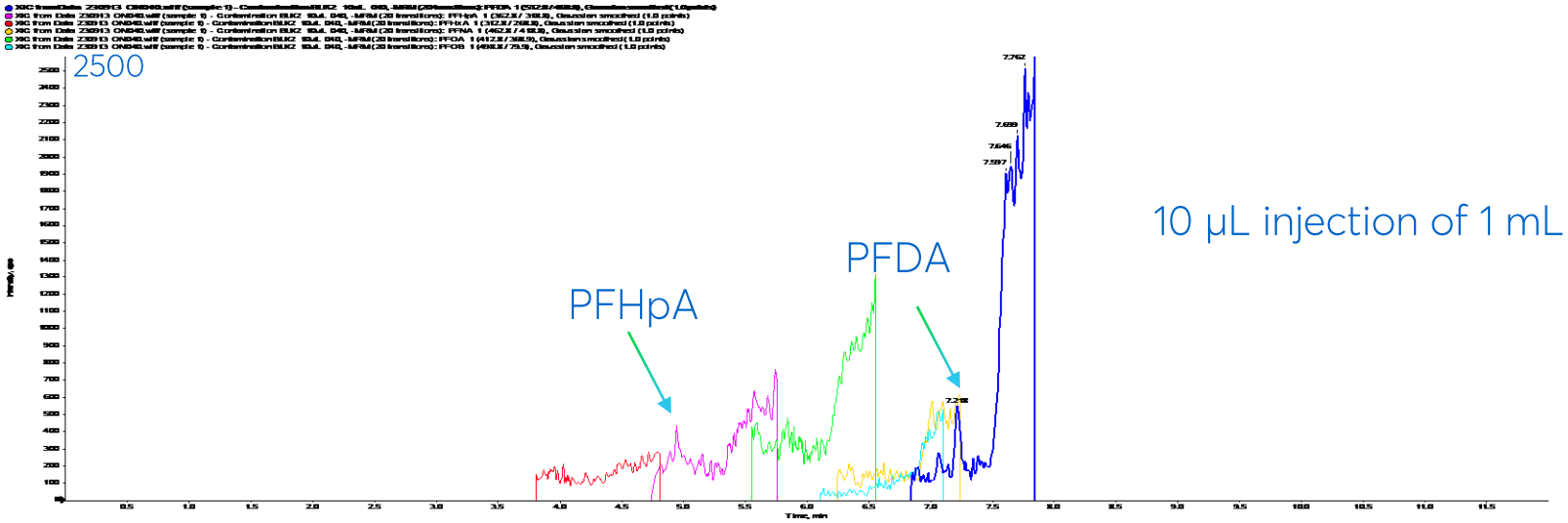
1 µL injection



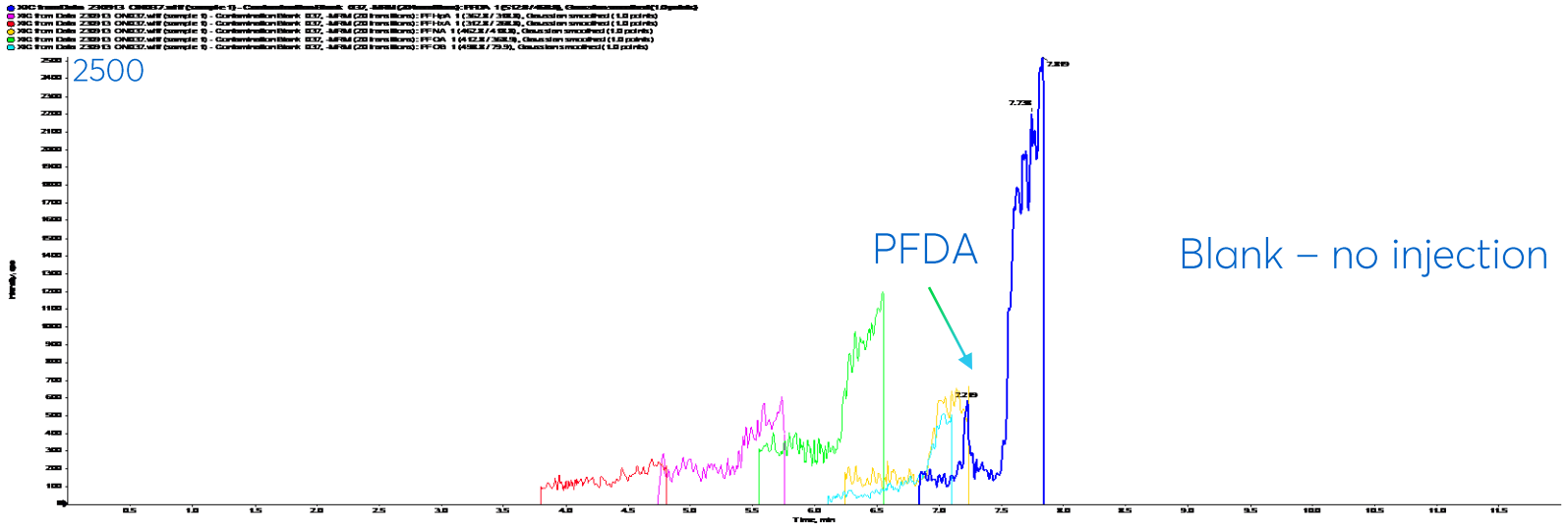
10 µL injection

- Similar PFAS profile observed to that obtained when no delay column was used.
- Solvent tubing is a significant source of system related PFAS.
- Switch tubing to PEEK

Tracking system contamination: Blanks



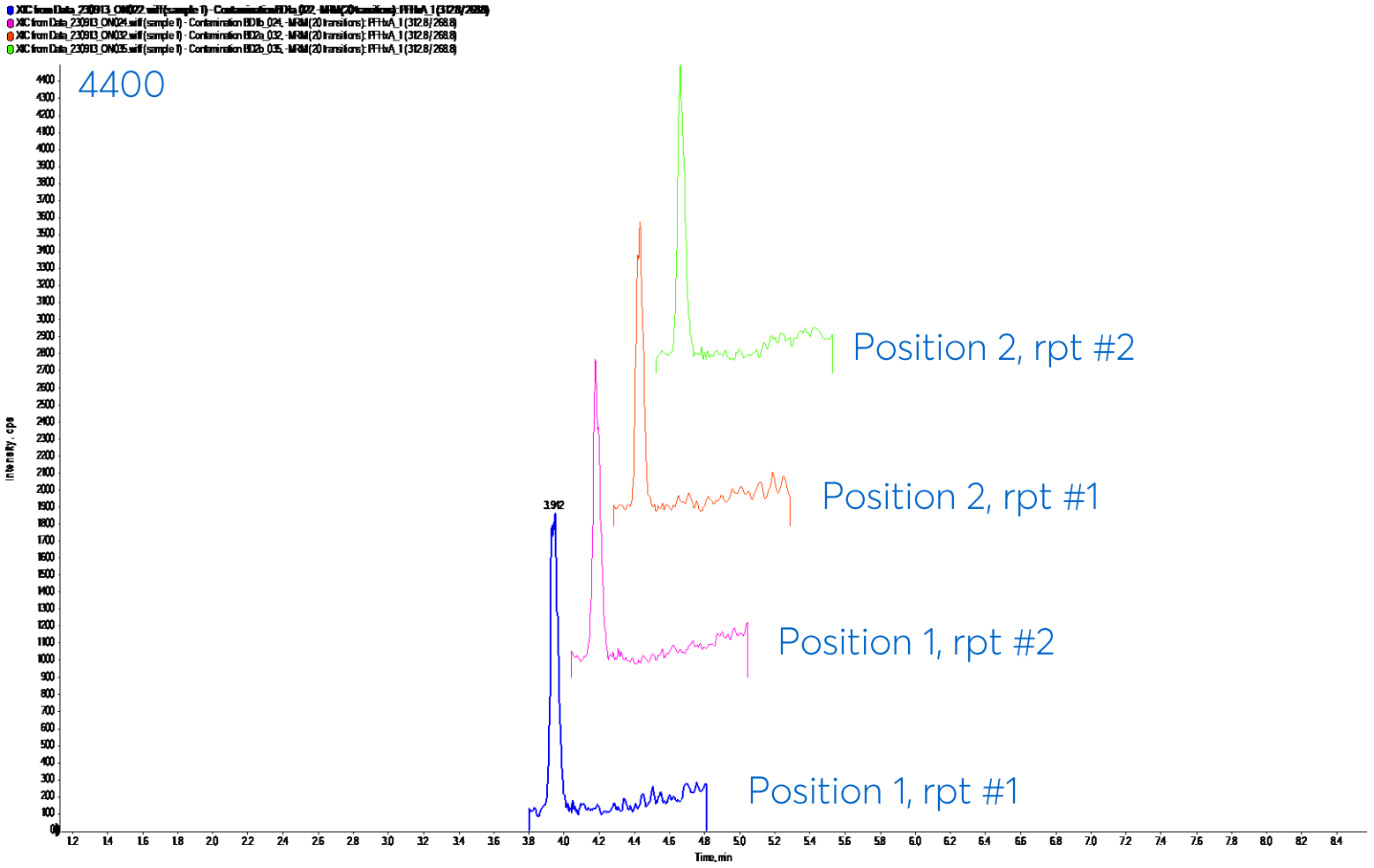
10 µL injection of 1 mL MeOH in transfer tube



Blank – no injection

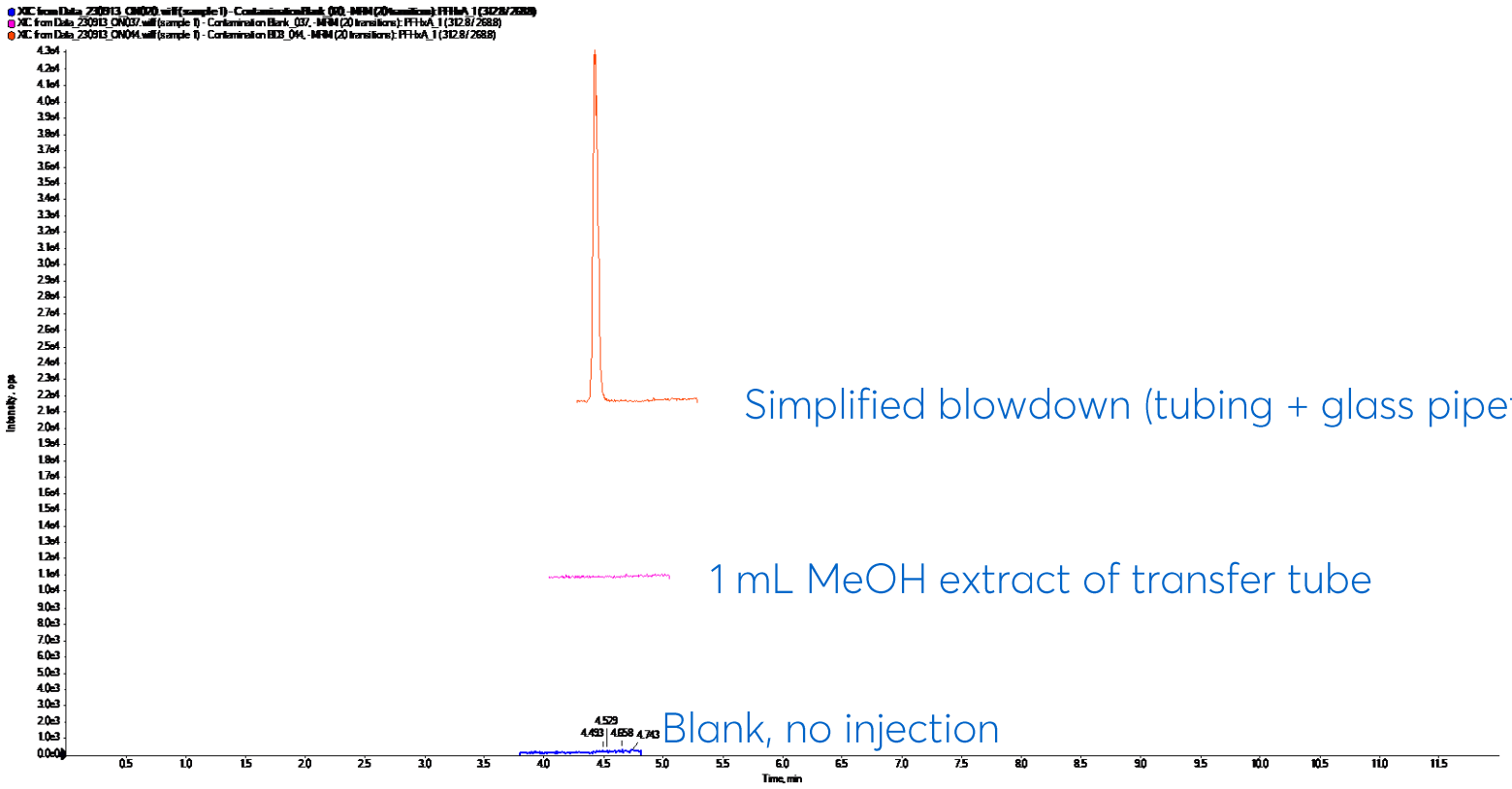
- Blanks are relatively clean.
- Conclusion – solvent tubing contributes significantly to system background PFAS levels

Sample Prep: N_2 Blowdown



- 8 mL MeOH blown down to dryness in 10 mL polypropylene transfer tube.
- N_2, 40L/min @ 60 °C
- Reconstituted in 1 mL MeOH
- All samples contaminate with PFHxA
- Level of contamination is consistent between positions
- Level of contamination consistent over time
- Originates from Blowdown rig or N_2 source?

Sample Prep: N_2 Blowdown



Simplified blowdown (tubing + glass pipette)

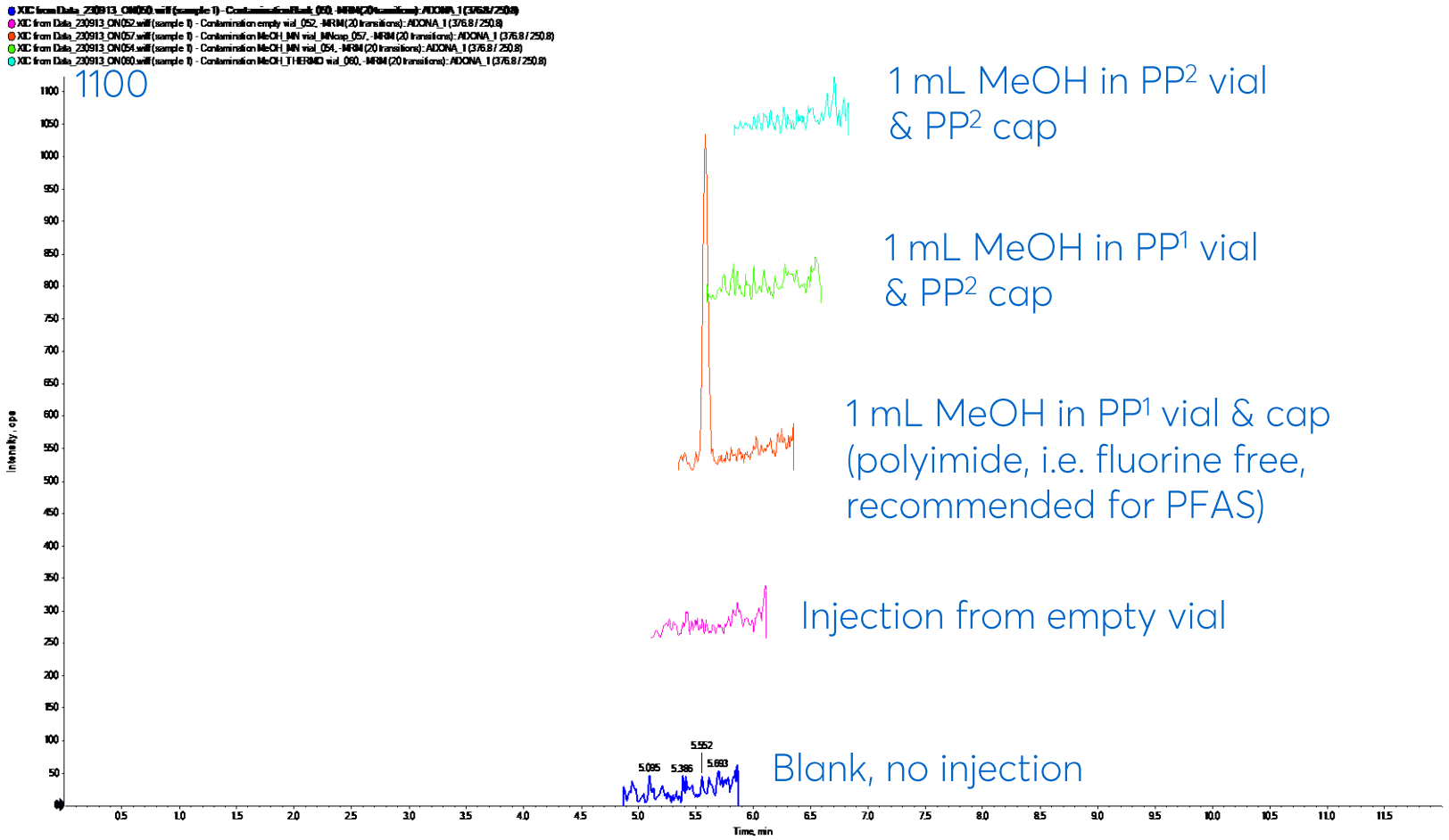
1 mL MeOH extract of transfer tube

Blank, no injection

- Blowdown rig was removed.
- N_2 direct from N_2 generator via polypropylene tubing and glass pipette
- No heat, so longer required to blow down, therefore increased signal!
- N_2 source appears to be the culprit.
- N_2 generator contains PTFE filters – potentially the source?
- Use activated carbon inline filter?

Sample Prep: Vials & caps matter!

- ADONA was observed in blank MeOH injections



1 mL MeOH in PP[2] vial & PP[2] cap

1 mL MeOH in PP[1] vial & PP[2] cap

1 mL MeOH in PP[1] vial & cap (polyimide, i.e. fluorine free, recommended for PFAS)

Injection from empty vial

Blank, no injection

Use PP vials & caps!

Polyimide cap is source

Cap or vial?

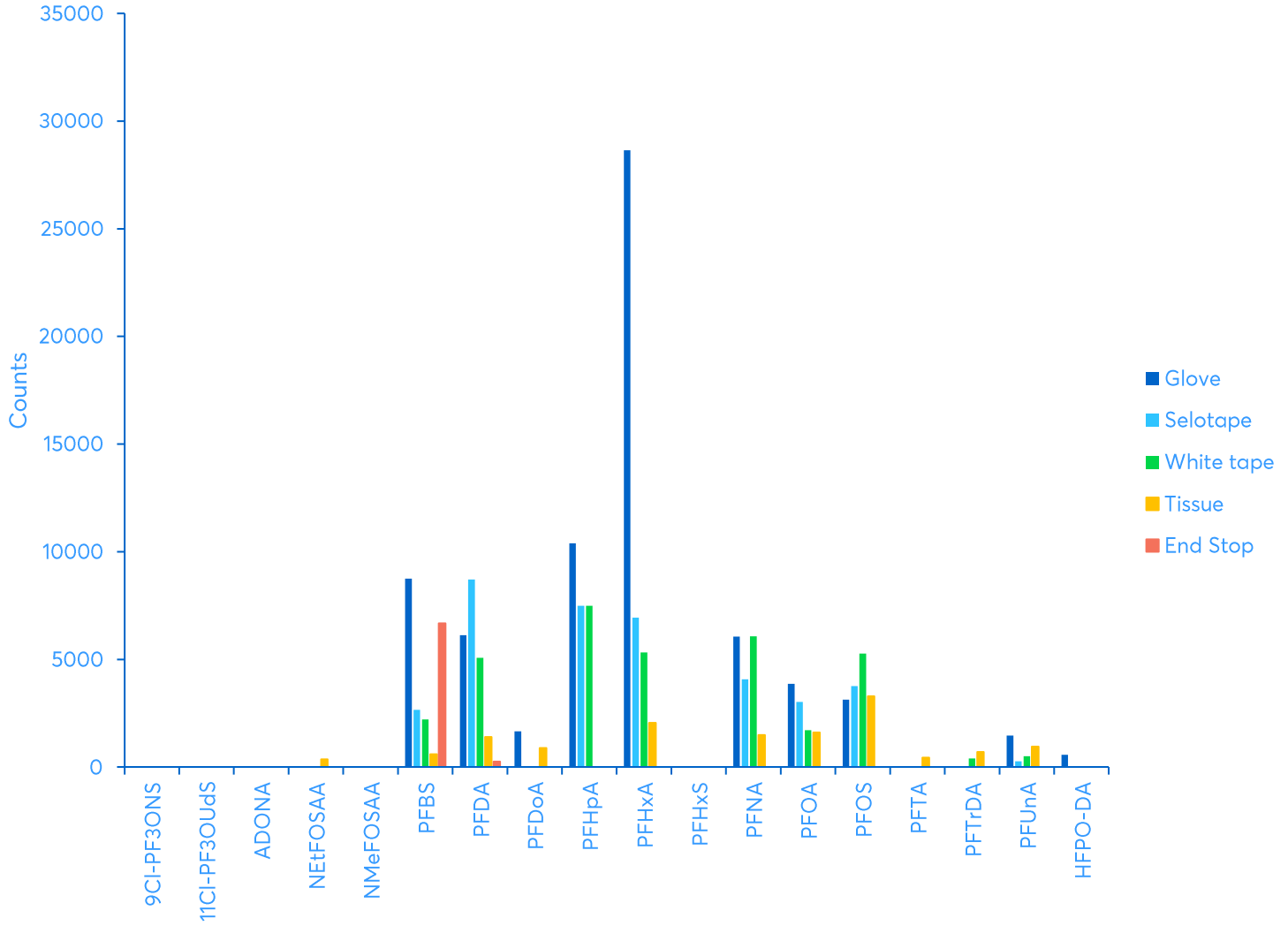
ALS OK

system OK

Testing sequence

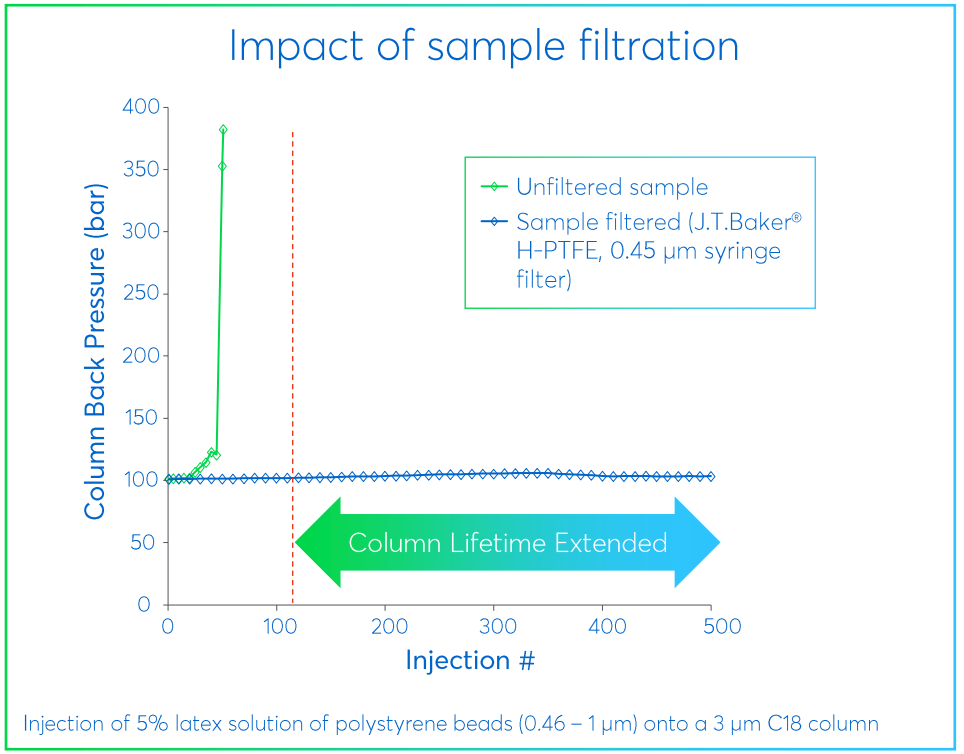
Other sources of contamination

- Samples of common laboratory consumables were extracted in 1 mL MeOH and analysed



Syringe Filters

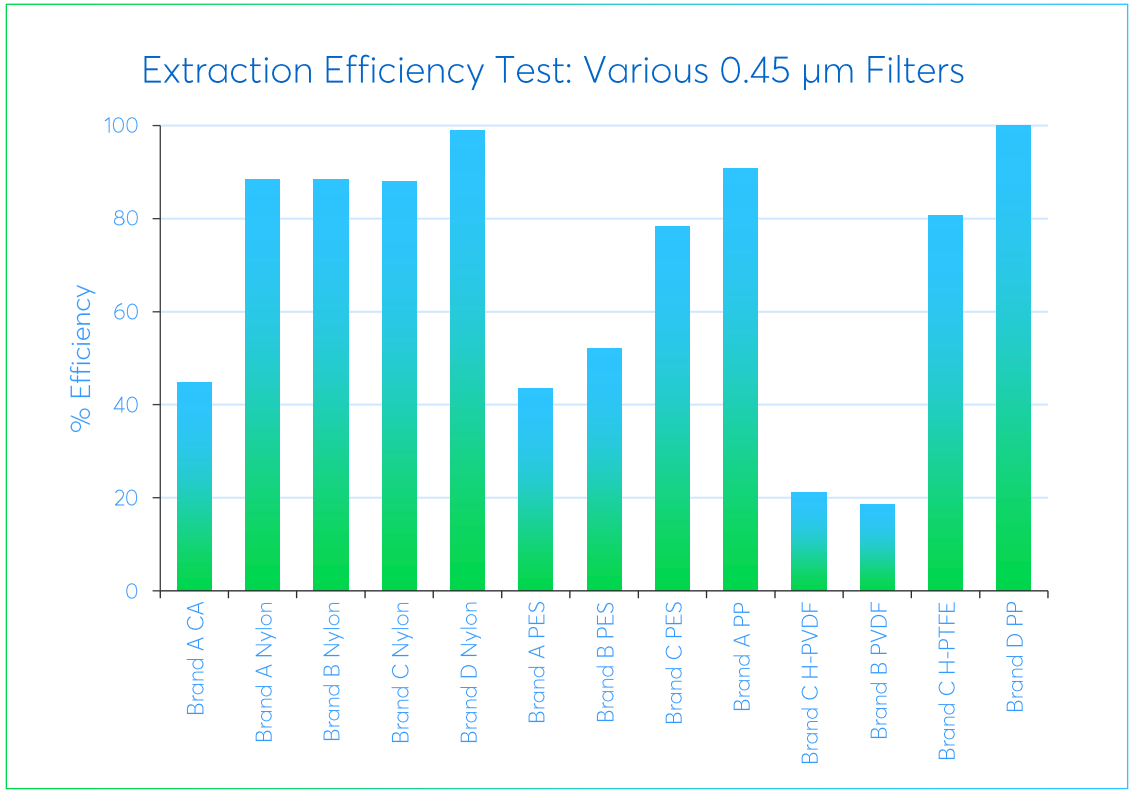
- Many samples contain particulates
  - Sub-micron particles → → → → large particles/micro-organisms
- Can damage LC system components & analytical column
  - Blockages
  - Peak distortion
  - Increase in back pressure
  - Reduced column lifetime
- Syringe filters provide fast, cheap & convenient solution

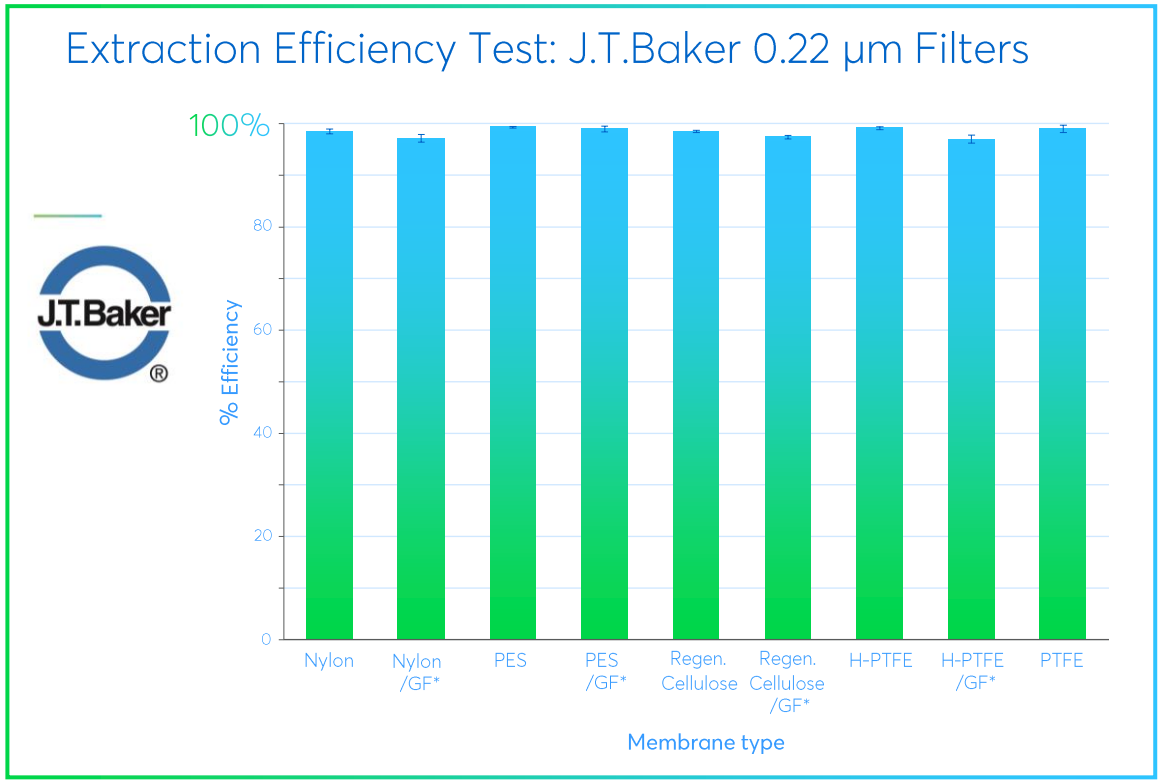


Injection of 5% latex solution of polystyrene beads (0.46 – 1 µm) onto a 3 µm C18 column

Filter quality

- Not all syringe filters are created equal!
- Important to use filters with high extraction efficiency

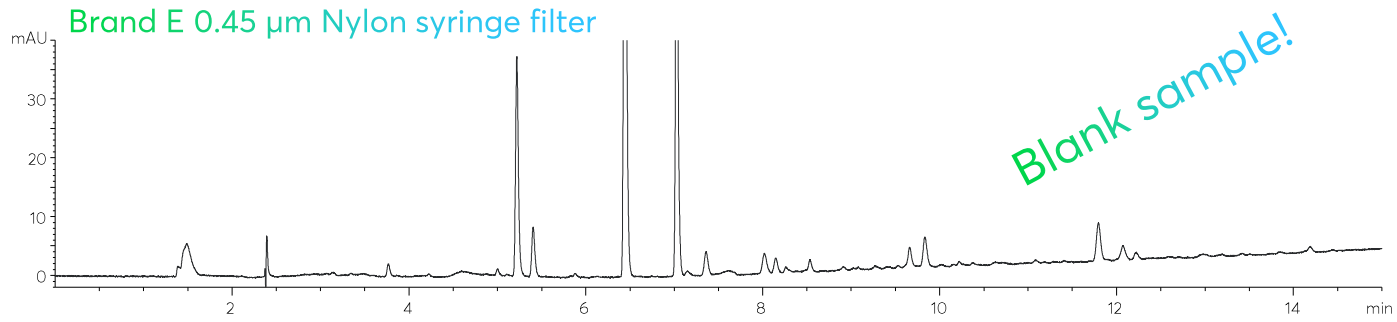


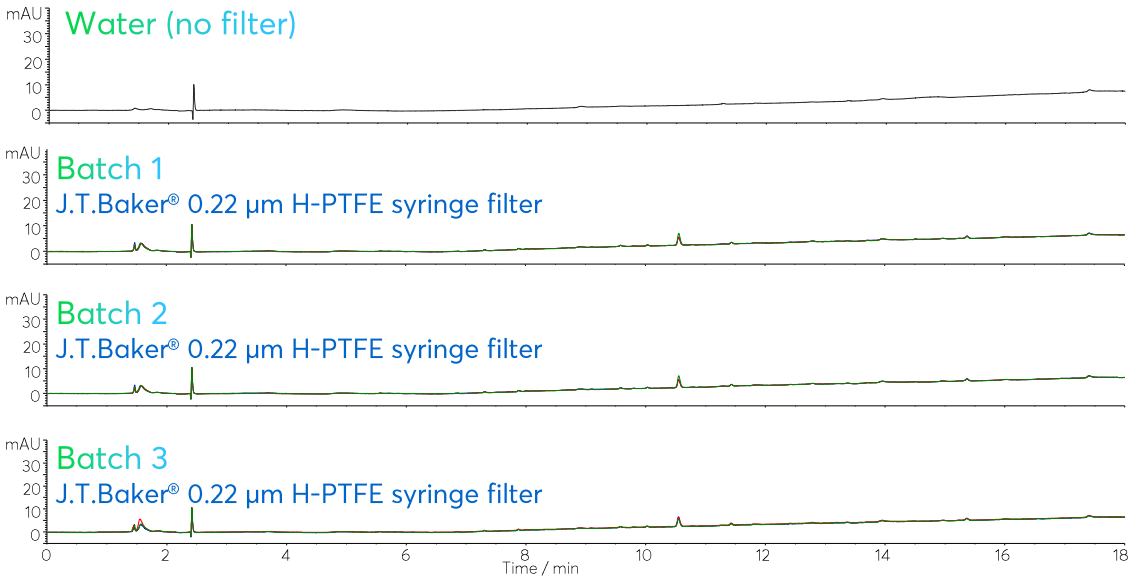


Extraction efficiencies determined by filtering a 0.01% latex solutions polystyrene beads through syringe filters by spectrophotometric analysis (UV, 272 nm). Triplicate analyses performed for multiple batches.

Filter quality: Extractables

- Poor Quality filters can contaminate samples
  - Ghost peaks
  - Interfere with target analyte quantification
- Using high quality syringe filters protects sample integrity.
- Filters easily tested for extractables by LC-UV:



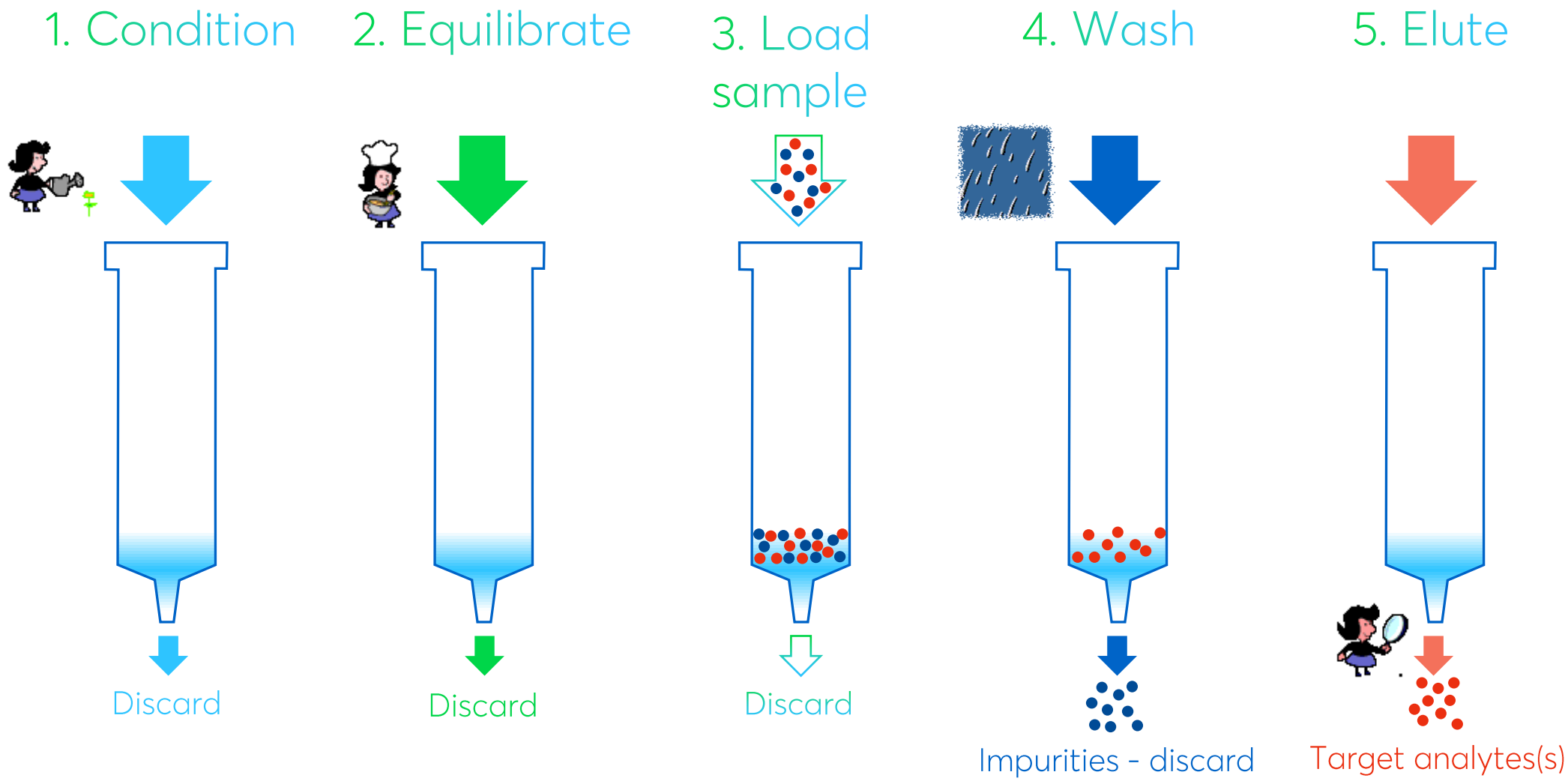


Method:

1 mL of H_2O or MeOH passed through filter.
Eluent analysed by HPLC

Column:	Avantor® ACE® 3 C18, 150 x 4.6 mm
Mobile phase A:	H_2O
Mobile phase B:	MeCN
Gradient:	5 to 100% B in 15 mins.
Flow rate:	1 mL/min
Temperature:	30 °C
Detection:	UV, 214 nm
Injection volume:	100 µL

SPE - Visualising the process (schematically):



Solid Phase Extraction (SPE)

- Method development required
  - Relatively easy to do
  - Many manual manipulation steps
- Sample preconcentration
  - Possible to preconcentrate >100 times
  - Method development requires some skill
  - Solvent usage less than liquid-liquid extraction
- Minimal issues with matrix as removes more of matrix than other techniques
- Applicable to a wide range of compounds with differing log D
- Excellent recoveries

Selecting a sorbent

Interaction Mode	Interaction Energy (kcal/mol)
Ionic Interactions	50 – 200
Polar Interactions	3 – 10
Non-polar Interactions	1 - 5

- Stronger retention offered by ion exchange
- Combination of interactions provides best for selectivity

SPE – Considerations for the Five-Stage Process

1. Condition
   - Strongly eluting solvent
   - Suitable volume
2. Equilibrate
   - Weakly eluting solvent
   - Suitable volume
3. Load
   - Ionization state
   - Concentration of analyte vs bed weight
   - Cleanliness of matrix
4. Wash(es)
   - Elutropic strength
   - Suitable volume
5. Elute
   - Elutropic strength
   - Suitable volume

Conclusion

01

- Introduction to PFAS

02

- Contamination issues with PFAS

03

- Use of delay columns

04

- Sample preparation techniques for environmental analysis

05

- Conclusions

06

- Acknowledgements

Acknowledgements

- Avantor colleagues: Gemma Lo, Arianne Soliven, Gemma Howse and Amelia Knight

Thank you

Anthony.edge@avantorscience.com

chromsupport@avantorsciences.com