SOLUTIONS BY LCTech

Automated Sample Preparation for Determination of Perfluoroalkyl and Polyfluoroalkyl Substances (PFAS)

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LCTech GmbH

History and Development

- 1998 Foundation LCTech GmbH

- 2000 First own developed system from LCTech
  - TACS totally automated clean-up system for pesticide sample preparation

  …
- 2011 Start of a new period in LCTech - FREESTYLE
  - Robotic system for automated sample clean-up SPE, EVA, GPC…
  - 2016 FREESTYLE XANA – Automated sample clean-up for water samples
  - 2019 FREESTYLE SPE (XANA)-PFAS – Dedicated material for PFAS application

Perfluoroalkyl and Polyfluoroalkyl Substances (PFAS)

Recent Activities Europe, EFSA 2018

SOLUTIONS BY LC Tech

- Following Stockholm Convention POP Regulation (EG) No 850/2004 for persistent organic pollutants, EU No 757/2010 for update of above, e.g. that restricts PFOS worldwide EU published in 05/2018 the restriction for production/distribution, trading and import of PFOA/ PFC with deadline for execution of 04.07.2020 (EU 2017/1000)

- Restriction will also be applied for all other matrix e.g. workman and other textiles with deadline of 04.07.2023

Perfluoroalkyl and Polyfluoroalkyl Substances (PFAS)

Recent Activities Europe, EFSA 2018

SOLUTIONS BY LC Tech

- New work for risk assessment PFAS
- First PFOS/PFOA (published in December 2018)
- Other PFAS: under development
- Based on human data, instead of animals

- EFSA recommendation after meeting of the CWG PFAS 16.10.2018; EURL/NRL-Workshop on 30 - 31 Oct 2018

- In comparison to the Health Based Guidance Values of EFSA of 2008:
  - → a factor of about 80 lower for PFOS (13 ng/kg bw/wk)
  - → a factor of about 1750 lower for PFOA (6 ng/kg bw/wk)

Perfluoroalkyl and Polyfluoroalkyl Substances (PFAS)

Work EFSA Mentioned in 2018 to Look into

SOLUTIONS BY LC Tech

- Mixture approach compared to TEQ like Dioxins?
- Extend to look at a certain number of PFAS? Which ones?
- Lower LOQs in case possible, especially for food regularly consumed
- Looking for hot spots:
  - Animals foraging outside
  - Also private owners of chicken farms …
- Bioassay to estimate contribution unknown PFAS by comparison with LC/MS

Perfluoroalkyl and Polyfluoroalkyl Substances (PFAS)

Recent Activities in the USA

SOLUTIONS BY LC Tech

- In November 2018, the United States Environmental Protection Agency (U.S. EPA) update a Review of Method US EPA 537.1:
  "Determination of Selected Per- and Polyfluorinated Alkyl Substances in Drinking Water by Solid Phase Extraction and Liquid Chromatography/ Tandem Mass Spectrometry (LC-MS/MS)"

- The method uses an offline solid-phase extraction (SPE) with (LC-MS/MS) to extract, enrich, and determine 18 PFAS in drinking water.

Perfluoroalkyl and Polyfluoroalkyl Substances (PFAS)

Recent Activities in the USA

SOLUTIONS BY LC Tech

Analyte[a]	Acronym
Hexafluoropropylene oxide dimer acid	HFPO-DA
N-ethyl perfluorooctanesulfonamidoacetic acid	NEtFOSAA
N-methyl perfluorooctanesulfonamidoacetic acid	NMeFOSAA
Perfluorobutanesulfonic acid	PFBS
Perfluorodecanoic acid	PFDA
Perfluorododecanoic acid	PFDoA
Perfluoroheptanoic acid	PFHpA
Perfluorohexanesulfonic acid	PFHxS
Perfluorohexanoic acid	PFHxA
Perfluorononanoic acid	PFNA
Perfluorooctanesulfonic acid	PFOS
Perfluorooctanoic acid	PFOA
Perfluorotetradecanoic acid	PFTA
Perfluorotridecanoic acid	PFTrDA
Perfluoroundecanoic acid	PFUnA
11-chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	11Cl-PF3OUdS
9-chlorohexadecafluoro-3-oxanone-1-sulfonic acid	9Cl-PF3ONS
4,8-dioxa-3H-perfluorononanoic acid	ADONA

Perfluoroalkyl and Polyfluoroalkyl Substances (PFAS)

Summary of the Method

SOLUTIONS BY LC Tech

- 250 mL water sample with added surrogates is loaded onto an offline SPE cartridge containing polystyrenedivinylbenzene (SDVB)
- Rinsing of bottle with MeOH, this rinsate applied for elution
- Concentration to dryness
- Adjusted to a 1-mL volume with 96:4 % (vol/vol) methanol:water
- addition of internal standards
- 10-µL injection into an LC- MS/MS equipped with a C18 column

Perfluoroalkyl and Polyfluoroalkyl Substances (PFAS)

QC-Requirements: Initial QC to prove Analytical Setup

SOLUTIONS BY LC Tech

- 9.2.2. Initial Demonstration of Low System Background
- 9.2.3. Initial Demonstration of Precision (IDP) of 4-7 replicates
- 9.2.4. Initial Demonstration of Accuracy (IDA)
- 9.2.5. Initial Demonstration of Peak Asymmetry Factor
- 9.2.6. Minimum Reporting Level (MRL) Confirmation
- 9.2.7. Calibration Confirmation

Perfluoroalkyl and Polyfluoroalkyl Substances (PFAS)

QC-Requirements: Ongoing Requirements for Labs

SOLUTIONS BY LC Tech

- 9.3.1. Laboratory Reagent Blank (LRB)
- 9.3.2. Continuing Calibration Check (CCC)
- 9.3.3. Laboratory Fortified Blank (LFB)
- 9.3.4. Internal Standards (IS)
- 9.3.5. Surrogate Recovery

Perfluoroalkyl and Polyfluoroalkyl Substances (PFAS)

SPE Clean-up – Challenges in Daily Use

SOLUTIONS BY LC Tech

- Heavy workload
- Standardization
- Reproducibility

high demand for automation of the SPE procedure

- Easy handling
- Intuitive programming
- Plannable deadlines
- Meeting low detection limits
- it needs to be ensured that there is No blind values for PFAS Analysis brought in by equipment

Perfluoroalkyl and Polyfluoroalkyl Substances (PFAS)

SPE – Solution in Daily Use

SOLUTIONS BY LC Tech

- FREESTYLE SPE PFAS or
- FREESTYLE XANA PFAS

→ Minimised fluoroplastic components

→ Constantly low PFAS background

→ robust automation for 24/7 operation

FREESTYLE

SOLUTIONS BY LC Tech

XANA for Automated SPE in PFAS Analysis

Automation based on FREESTYLE BASIC

SOLUTIONS BY LC Tech

Valves, syringe pump & others
on top of y-axis (reduces dead volume in tubing)

xyz-robotic system

Flexible robotic area; the appropriate „area" can be created at any time by choosing the right racks

Rinsing port for needle

XANA for Automated SPE in PFAS Analysis

Easy Handling – Your Labware as Usual

SOLUTIONS BY LC Tech

Just hook the rack into the toothed plate.

XANA for Automated SPE in PFAS Analysis

Application of SPE Cartridges

SOLUTIONS BY LC Tech

Flexibility
just by adapters

XANA for Automated SPE in PFAS Analysis

Easy Handling – Simple Operation

SOLUTIONS BY LC Tech

- For samples > 100mL FREESTYLE XANA is applied
- 24 positions for 250 up to 1 L sample bottles
- Rack is easy to move and load
- Definition of *Analysis Batch* in US EPA 537.1 – a set of samples that is analysed on the same instrument during a 24-hour period, including no more than 20 Field Samples

XANA for Automated SPE in PFAS Analysis

Easy Handling – Simple Operation

SOLUTIONS BY LC Tech

- Sample bottles with special designed cap for one-hand operation.
- Angular position of the suction capillary to maximize the sample load.
- Suction capillary can be height-adjusted

XANA for Automated SPE in PFAS Analysis

Automated Rinsing of Sample Bottles

SOLUTIONS BY LC Tech

- Subsequently to sample loading Rinsing of the sample bottle and quantitative sample transfer by separate rinsing capillary is possible with up to 8 different solvents
- Fully automated
- Rinsing solution can also be applied after drying of columns e.g. to directly use this as eluting solvent

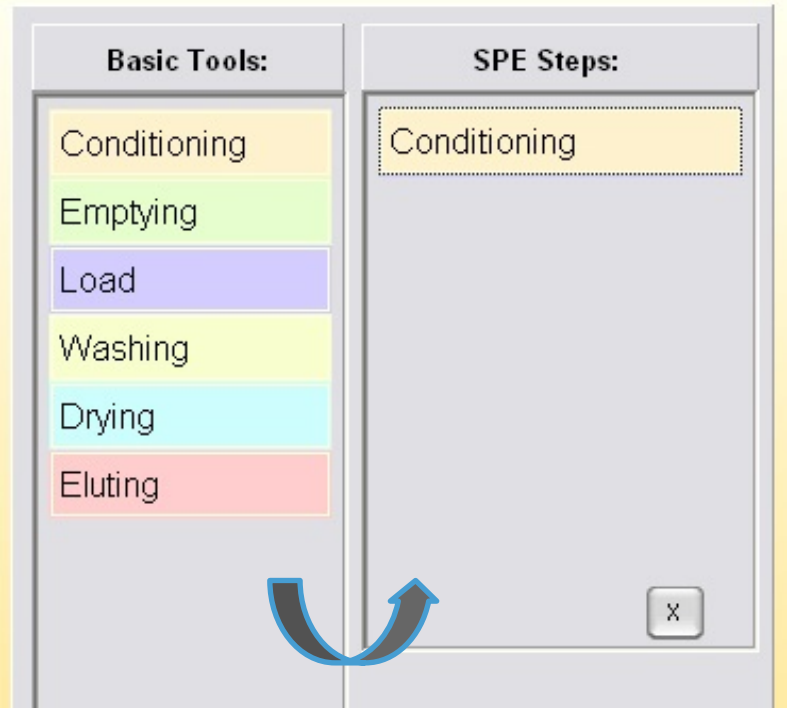
XANA for Automated SPE in PFAS Analysis

Simple Operation – Intuitive Software

SOLUTIONS BY LC Tech

Generate any SPE Method:

- Move next step from the basic tools with **drag & drop** to second column.
- The default button remains in the basic tools and can be chosen again.
- The collected steps in the second column build the method .
- Each button there can be individually changed and adopted by using the „bars".

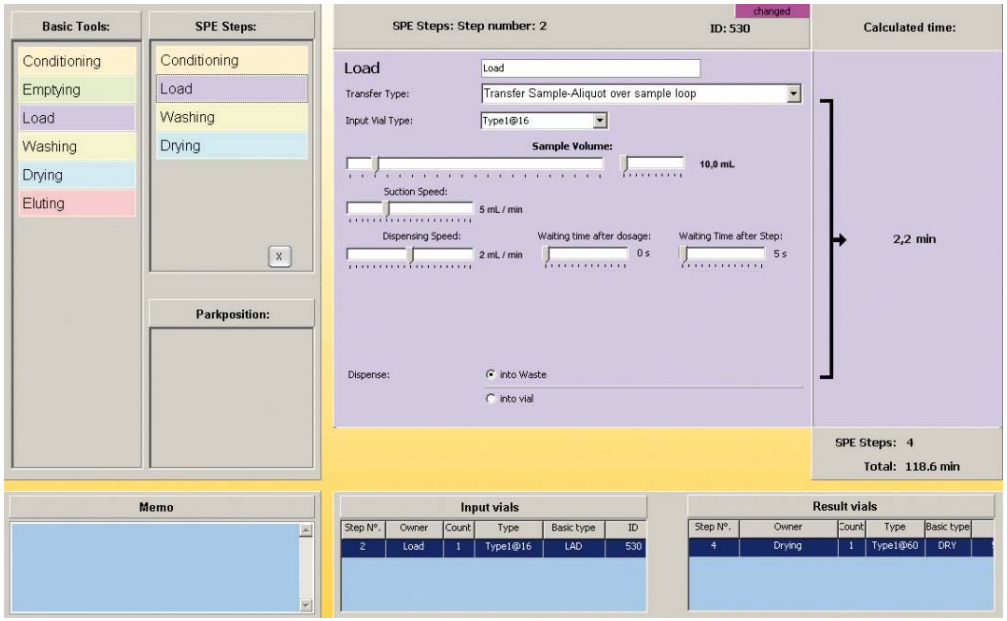


XANA for Automated SPE in PFAS Analysis

Simple Operation – Intuitive Software

SOLUTIONS BY LC Tech

Software allows the transfer of nearly any manual SPE method straight to the automation



XANA for Automated SPE in PFAS Analysis

Throughput- External Working Station

SOLUTIONS BY LC Tech

- polypropylene transfer tube system acc. to material chapter of the method
- Pumps 3 samples in parallel with flow rates: 1 – 30 mL/min.
- Conditioning, Washing, Rinsing and Drying of 3 columns in parallel with Up to 8 solvents for conditioning, rinsing and washing
- Detection of empty bottles, positions that are not taken aren't processed!

XANA for Automated SPE in PFAS Analysis

Workstation ensures parallel Loading

SOLUTIONS BY LC Tech

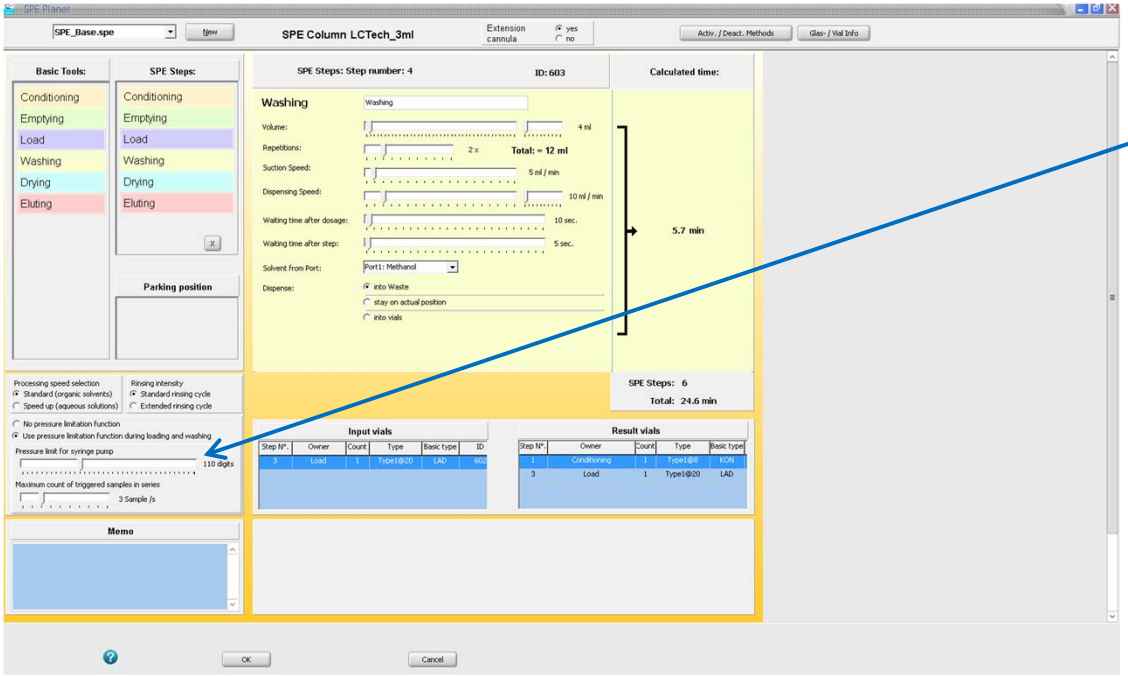
- Loading with positive pressure
- Tolerates back pressure up to 4 bar
- Batches of 3 columns processed at a time. 6 positions for loading, drying or eluting of columns (parallel loading of 3 samples and drying of 3 samples)

XANA for Automated SPE in PFAS Analysis

Planable Results – Meeting Timelines

SOLUTIONS BY LCTech

Non-stop policy – this is an unique error handling



Pressure Monitoring

Once this monitoring alerts a blocked cartridge, this one will be taken out of the sequence (noted in report) and the rest of the sequence processed

XANA for Automated SPE in PFAS Analysis

Study with FREESTYLE XANA for US EPA 537.1

SOLUTIONS BY LC Tech

- Samples
  - Laboratory Fortified Blanks (LFB) – purified water spiked with targets and surrogates with 4 ng/L (3 replicates)
- Standards:
  - Internal standard mix (not required for sample spiking) (Wellington PN EPA-5371S)
  - Surrogate mix (Wellington PN EPA 537SS-R1)
  - PFAS native mix (Wellington PN EPA-537PDS-R1)
- SPE Clean-up: Bond Elut LMS 500 mg, 6 mL (part number 12255021)
- Clean-up performed at LC Tech GmbH Germany on FREESTYLE XANA
- Analysis performed at Agilent Inc. Wilmington on LC-MS/MS

Automated SPE applied in acc. to US EPA 537.1

Application of US EPA 537.1 - labware

SOLUTIONS BY LC Tech

Collection of eluates in conical polypropylene tubes acc. to list of material in method

Automated SPE applied in acc. to US EPA 537.1

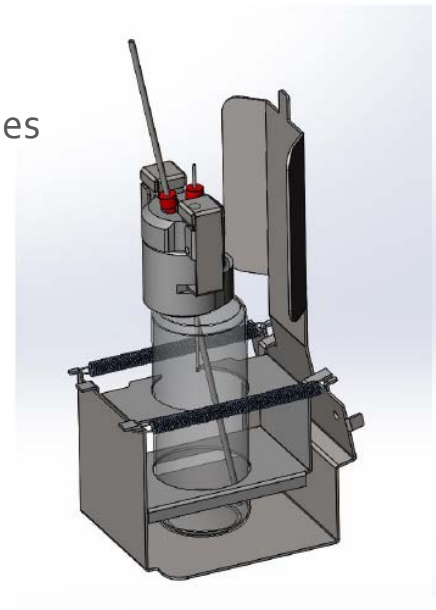
Application of US EPA 537.1 - labware

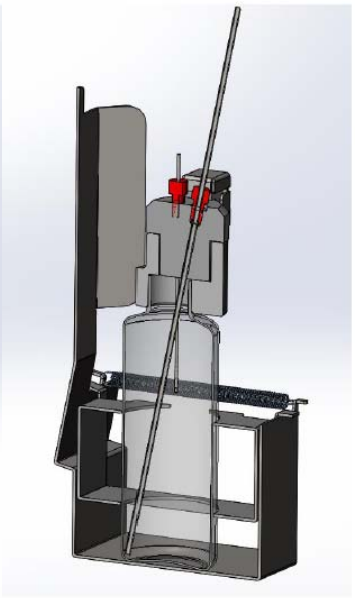
SOLUTIONS BY LC Tech

From US EPA Note: Samples and extracts should not come in contact with any glass containers or pipettes as these analytes can potentially adsorb to glass surfaces -

Material:

250mL polypropylene bottles





Automated SPE applied in acc. to US EPA 537.1

Application of US EPA 537.1

SOLUTIONS BY LC Tech

- Adapter for 6 mL Cartridge
- Applied here:
  - Bond Elut LMS 500 mg, 6 mL (part number 12255021)
  - EPA methods 537/537.1 were validated using this cartridge

Automated SPE applied in acc. to US EPA 537.1

Analytical Set-up

SOLUTIONS BY LC Tech

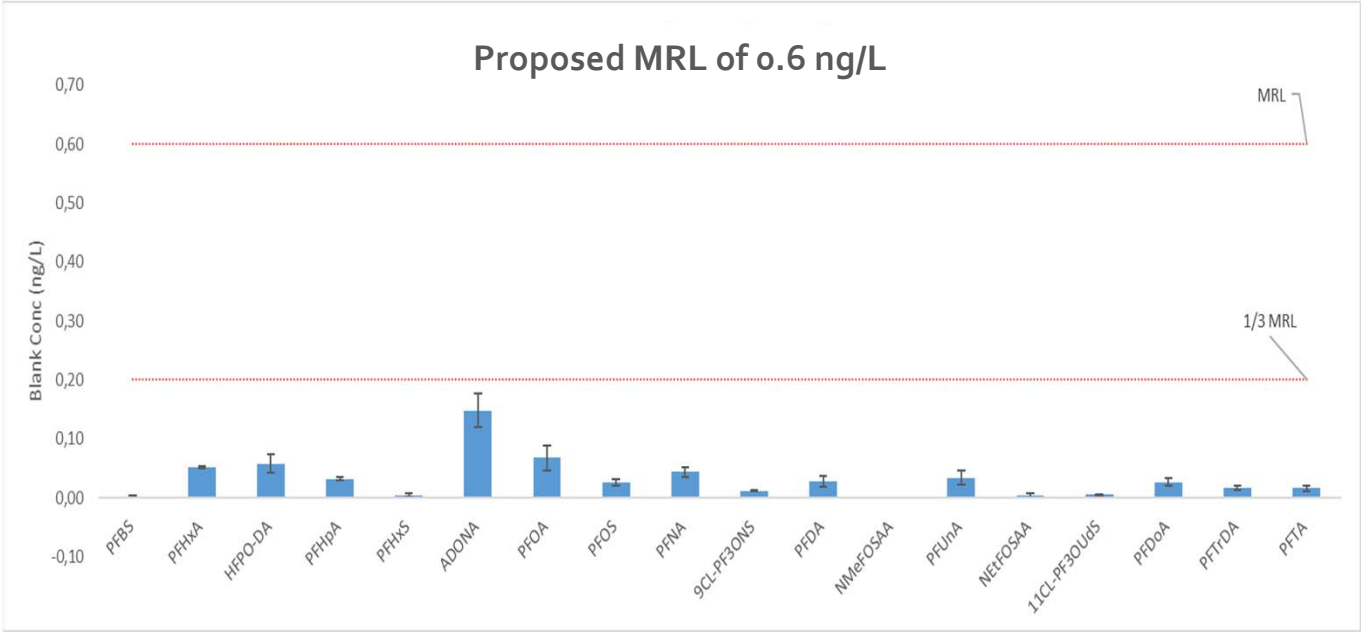
- LC/MS/MS: HPLC Instrument: Agilent 1290 Infinity II
- Delay column: Agilent InfinityLab PFC Delay Column (the purpose of this column is to separate potential PFAS contamination in the solvents, tubing, seals, etc. from the injected analytical peaks. This is commonly used for all PFAS analysis by LC).
- Analytical column: Agilent Zorbax RRHD EclipsePlus C18, 2.1 x 100 mm, 1.8 µm (part number 959758-902).
- Mass Spectrometer: Agilent 6470 Triple Quadrupole LC/MS/MS with Agilent Jet Stream Ion Source

Automated SPE applied in acc. to US EPA 537.1

Demonstration of Low Background

SOLUTIONS BY LC Tech

All analytes to be below 1/3 the lowest Standard; Proposed MRL of 0.6 ng/L



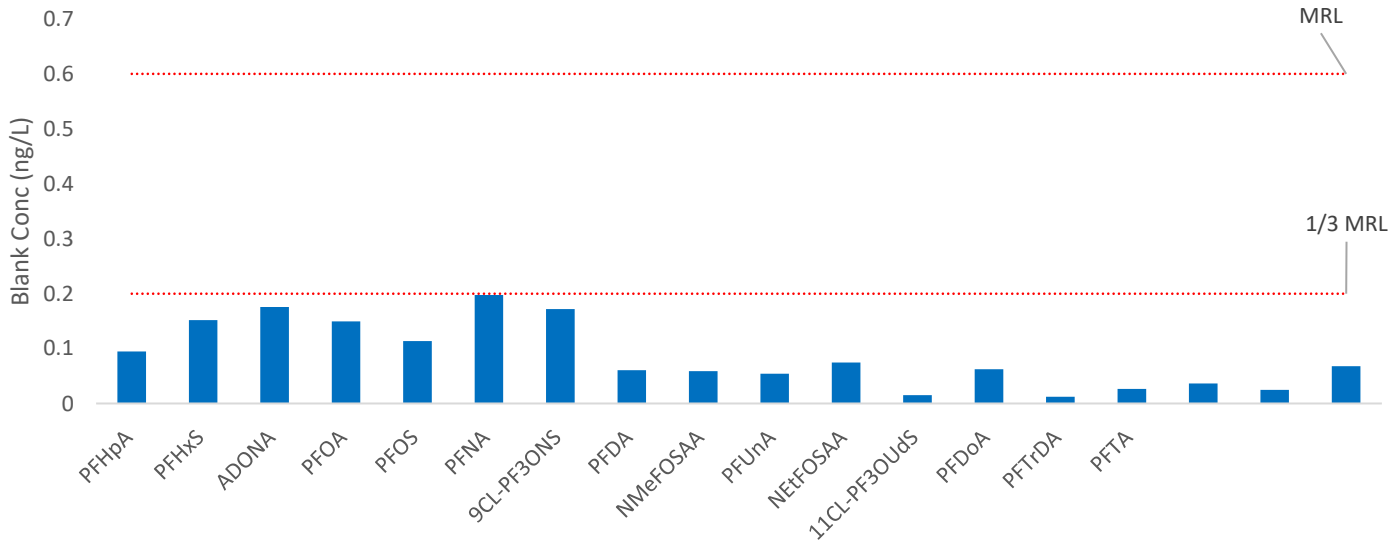
Automated SPE applied in acc. to US EPA 537.1

Additional Info Demonstration of Low Background

SOLUTIONS BY LC Tech

Despite the values are according to requirement, check for the cause of the contamination by evaporating all solvents offline.

Evaporation Experiment of the sum of all solvents in the protocol without FREESTYLE involved

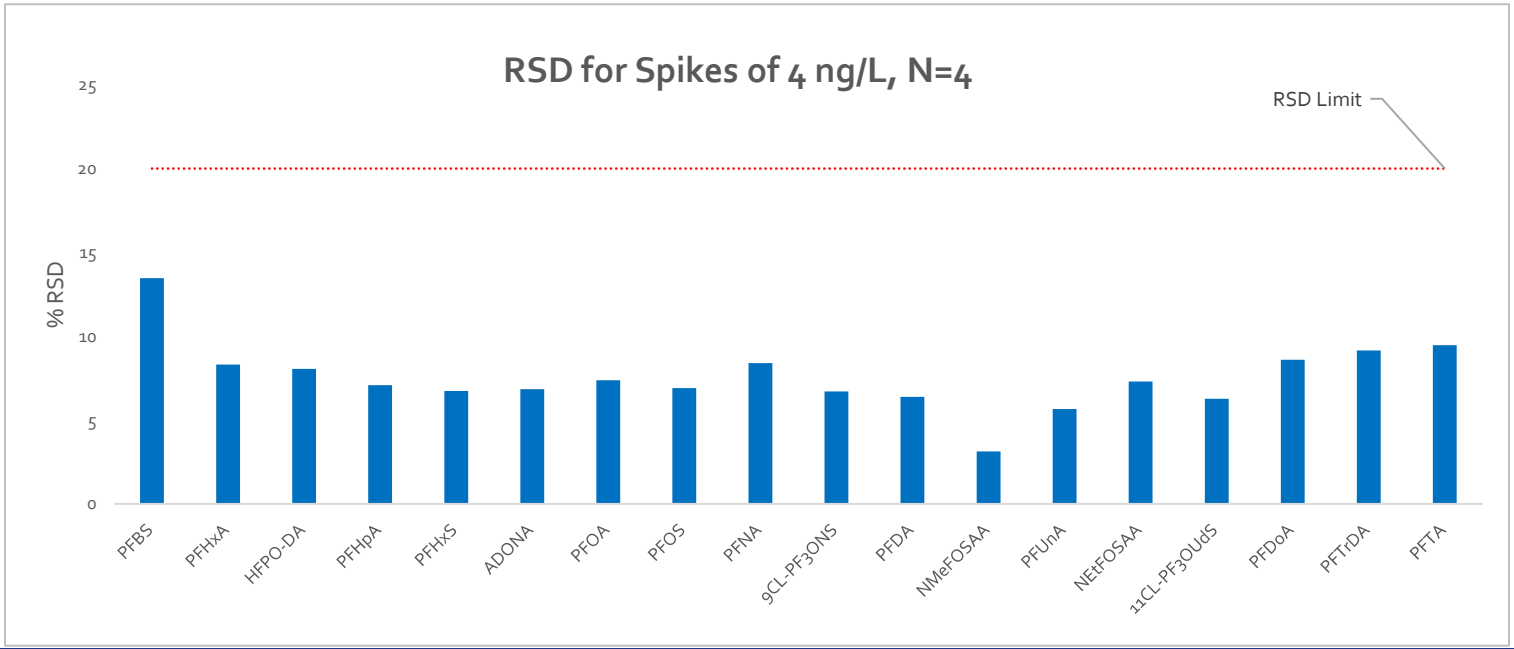


Automated SPE applied in acc. to US EPA 537.1

Initial Demonstration of Precision

SOLUTIONS BY LC Tech

Analyse 4 to 7 replicate LFB fortified near the midrange calibration whereof the Relative Standard Deviation RSD within must be <20%

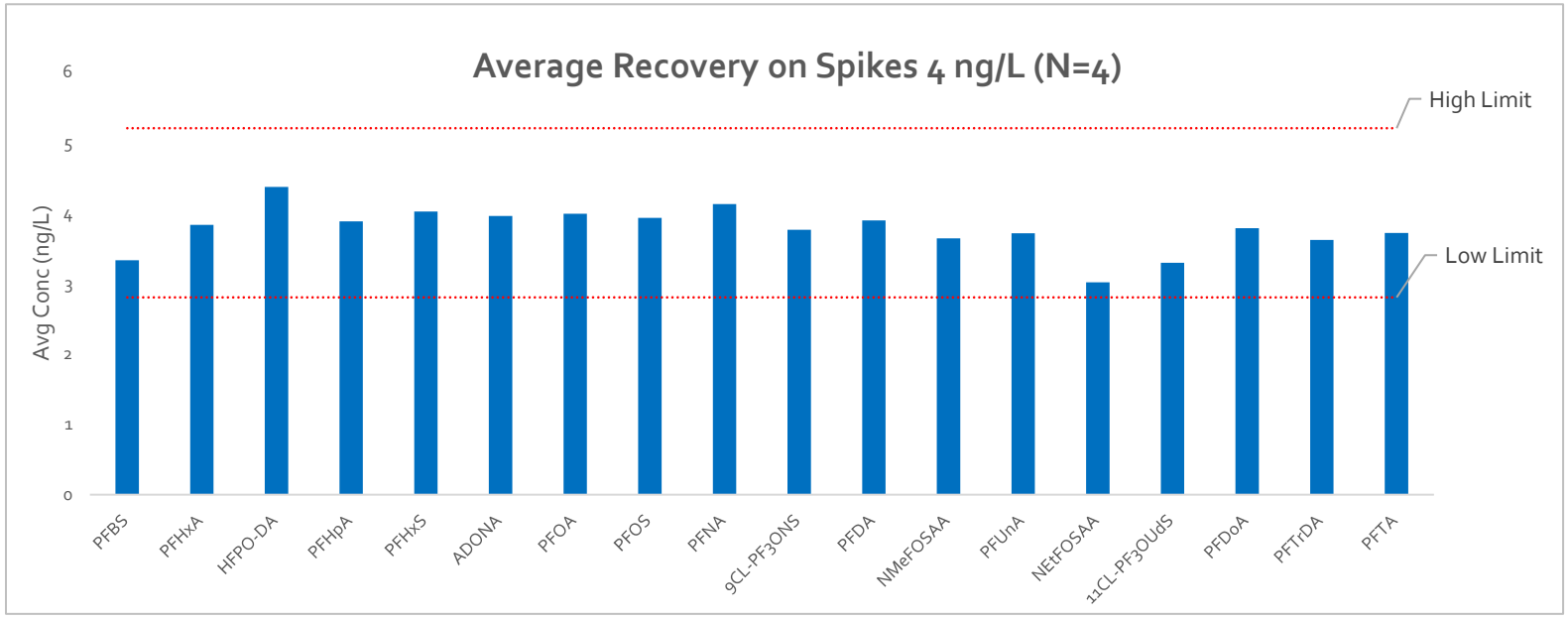


Automated SPE applied in acc. to US EPA 537.1

Initial Demonstration of Accuracy IDA

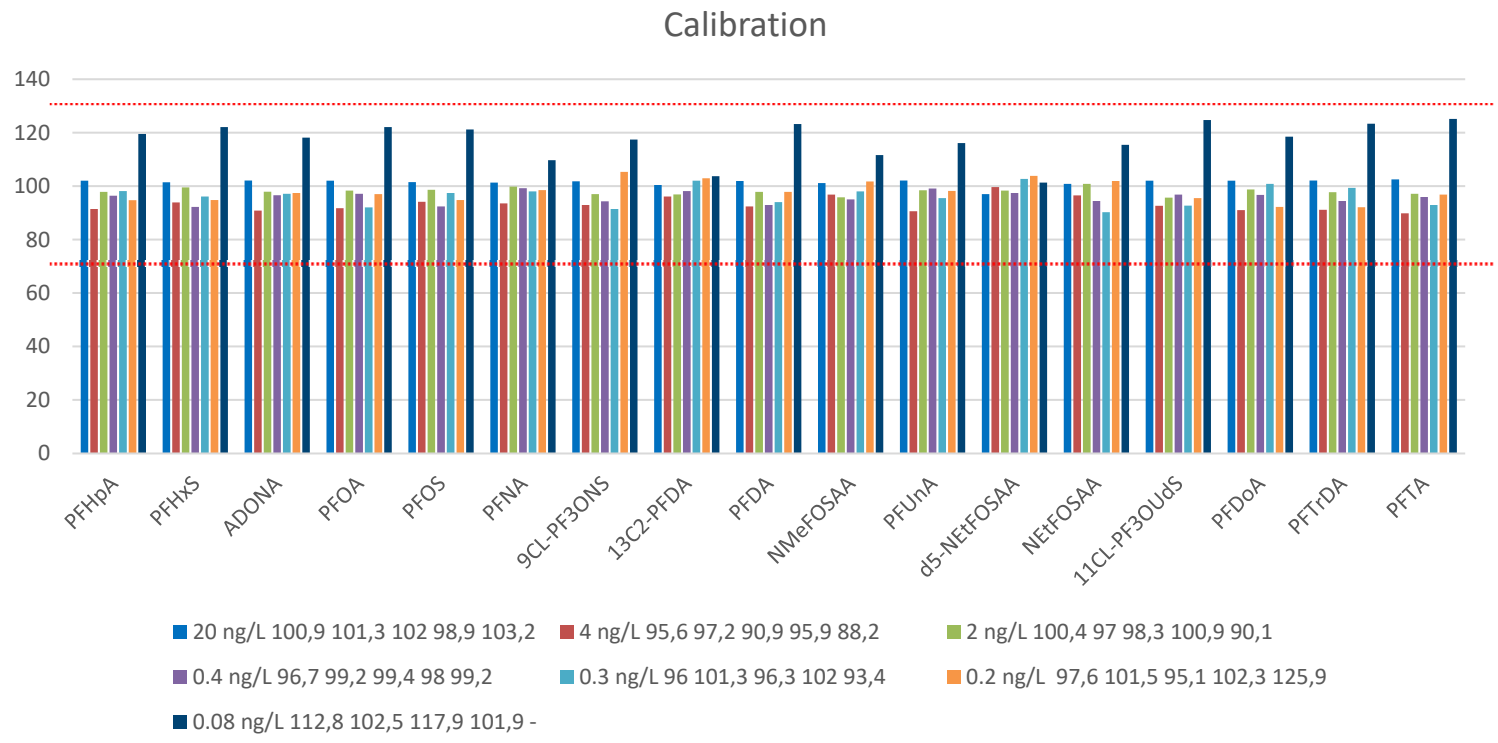
SOLUTIONS BY LC Tech

Calculate average recovery for replicates used in IDP whereof the average recovery to be ± 30% of true value



Automated SPE applied in acc. to US EPA 537.1

Initial Calibration



Recoveries must be within 70 to 130% for all targets and surrogates with the lowest standard within 50 – 150%

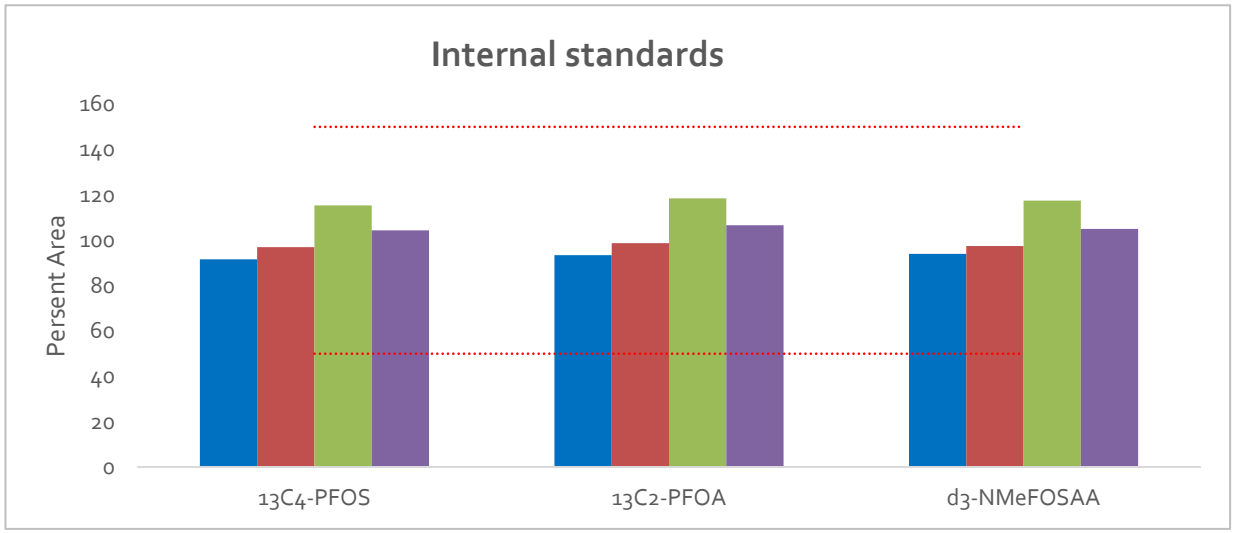
At leats 5 calibration points - here
All compounds calibrated with 1st order curve, forced zero intercept, and 1/(concentration) weighting.

Automated SPE applied in acc. to US EPA 537.1

Internal Standard IS

SOLUTIONS BY LC Tech

Compare continuously internal standard peak area to average response in calibration standards. Peak areas must be within ± 50 % of the average areas in the calibration standards.

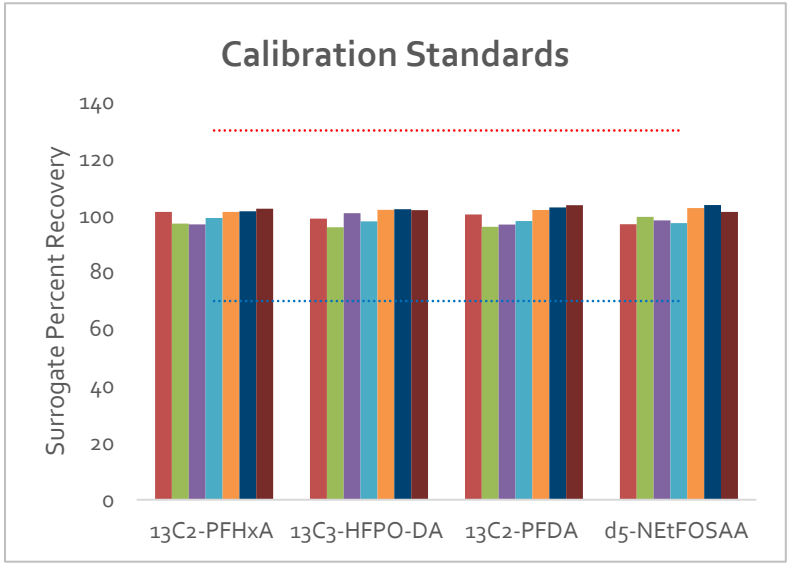


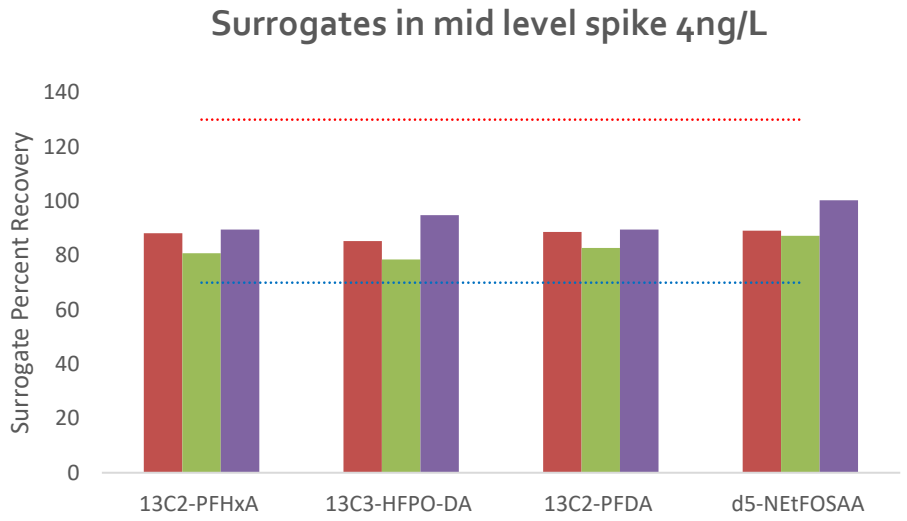
Automated SPE applied in acc. to US EPA 537.1

Surrogate Standard Recoveries SUR

SOLUTIONS BY LC Tech

Compare Surrogate Standard Recoveries which are added to CCCs, LRBs, LFBs, LFSMs, LFSMDs, FD, and FRB prior to extraction. Recoveries must be within 70 % to 130 %





Automated SPE applied in acc. to US EPA 537.1

The Automated Solution

SOLUTIONS BY LC Tech

- FREESTYLE XANA as automated sample delivery system in combination with material listed in chapter 6 and SPE cartridge Bond Elut is a working system that is applicable for US EPA quality objectives for water analysis on PFAS following US EPA 537.1.

- Automation of SPE helps to standardize, increase reproducibility, and meeting timelines thus ensuring to avoid blind values for PFAS Analysis

- FREESTYLE XANA means full automation without any manual interaction from loading an extract to the eluted sample of up to 24 samples in one batch

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Thank you for Your Attention!

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