AUTOMATED SAMPLE PREPARATION FOR THE DETERMINATION OF PERFLUOROALKYL AND POLYFLUOROALKYL SUBSTANCES (PFAS) IN DRINKING WATER, ACCORDING TO US EPA 537.1 AND EUROPEAN REGULATIONS

The topic of PFAS is more urgent than ever. The latest EU Drinking Water Directive with amendments regarding PFAS was set in place on 12 January 2021. Also in the USA, the corresponding regulation US EPA 537.1 for PFAS in drinking water was recently updated.

Nowadays, the analytical procedures for the determination of PFAS are not yet as harmonised and regulated as the procedures for other substances, like PCDD/F or PCB. Not only is the final determination of PFAS with LC/MS-MS methods is very demanding, but so, too, is the sample preparation, regardless of regional regulations. The samples are purified and enriched by solid-phase extraction, eluted in a significantly smaller volume in methanol and then concentrated to dryness without loss before being redissolved.

For a standardized and automated sample preparation in PFAS analysis, LCTech's Freestyle SPE system, in combination with the vacuum concentrator D-EVA, allows a specially adapted automated concentration of the substances.

In order to be able to achieve the requested limits of determination for the new MRL values, a sufficiently large sample-volume must be applied. Such large sample volumes result in a very high workload and time requirement for manual SPE, which is why automated processing of the samples, besides standardization, is advantageous as well. Another major challenge is posed by the ubiquitous existence of perfluorinated and polyfluorinated substances in the laboratory. The contamination of PFAS analytes in the samples must be kept as low as possible in order to avoid false-positive results. An undesirable, well-known source of PFAS in samples is the fluorocarbon materials, which is used commonly in laboratory equipment and materials, such as PTFE. These release small amounts of PFAS during contact with the sample, which leads to significantly increased background levels. Therefore, in the Freestyle SPE-PFAS system for automated processing of the SPE protocol, all fluorocarbon materials have been consistently minimised and replaced by polyethylene or polypropylene.

Both environmental samples, such as soil and water, and biota samples, such as animal tissue or food, can be purified automatically with the Freestyle SPE-PFAS system. If sample volumes of more than 100 mL are inserted, the system is upgraded with the XANA water module. This allows up to 24 samples of 1 L to be pumped from the rack via the SPE cartridge and significantly increases sample-throughput by simultaneously processing up to 7 samples.

Figure 1 shows the Freestyle-PFAS-XANA system with the capacity of up to 24 1 L bottles (in the figure, glass bottles) with a D-EVA in use. To implement US EPA 537.1, the samples were placed in 250 mL PE bottles and the eluate was collected in 15 mL or 50 mL Falcontubes™. Without further sample transfer of the eluate, the samples in these vessels are then inserted in the appropriate rotor of the D-EVA for gentle evaporation to dryness.

For evaluation, the PFAS compounds of the existing DIN 38407-42 and the analytes of the updated US EPA 537.1 were processed on a Freestyle PFAS XANA system and evidence of all formal requirements for validation for US EPA 537.1 were given. The results prove that all PFAS compounds are recovered well, that no traces adhere to the system and at the same time no blank values are generated during processing on the robot. For this experiment, the PFAS background-values of pure solvents were compared with the values of blank samples and a selection shown in table 1.

Following SPE purification, the eluate of 8 mL methanol is evaporated to dryness and almost dryness and redissolved for analysis in methanol/water 96:4 (v/v) according to US EPA 537.1.

Even this seemingly unproblematic step poses challenges for laboratories in PFAS analysis. Adhesion of PFAS to glass walls makes most of the equipment typically used for concentration unsuitable. Consequently, the only option remaining would be the time-consuming and costly supervised blow-off with nitrogen, which is aimed to be avoided due to unintentional aerosol formation.

LCTech provides an automated solution in a D-EVA apparatus, which holds the SPE eluates in common Falcontubes™ and performs a controlled process. By design, the D-EVA avoids losses, as it uses infrared light for heating, lowers the boiling temperature with specially low pressure gradient, and prevents aerosol formation by its rotation. Additionally, as neutral PFAS in particular tend to be lost when concentrating beyond the dry point, it is necessary to ensure that the protocol is terminated in a controlled manner in time. For this purpose, the D-EVA has a special sensor in a reference vessel, which reliably detects the dryness reached and stops the process. For this purpose, in-house protocols have been developed with optimal rotational speed and related termination temperature. When the target point is reached, the centrifuge chamber is ventilated and the infrared light used for temperature control is switched off so that no further direct heating power is applied to the sample vessels. The so-called 'memory effect', known from heating blocks and water baths, is thus avoided. In this way, even the neutral PFAS can be concentrated very quickly and measured with comparably high recoveries compared to the cost- and time-intensive nitrogen blow-off.

The graph in figure 2 shows the recovery rates of various PFAS related to US EPA 537.1. Where 8 mL methanol were spiked and evaporated at the medium calibration level. The method blow-off under N2 in a water bath is compared to the evaporation method

Fig.1 : The Freestyle PFAS XANA unit system in combination with a D-EVA from LCTech

"methanol" in the D-EVA. The evaporated tubes are redissolved in methanol/water 95/4 plus internal standard and measured with HPLC-MS/MS.

Applying US EPA 537.1 precisely, a certain residual water

Table 12: Comparison of PFAS background concentrations of neat solvent and water samples processed with the FREESTYLE XANA system

Component Name	Background FREESTYLE [ng/mL]	Background Solvent [ng/mL]
PFBA	0	0
PFPeA	0,09	0,06
PFHxA	0,02	0,04
PFHpA	0,02	0,02
PFOA	0,05	0,05
PFDA	0,01	0,01
PFNA	0,01	0,01
PFBS	0	0
PFHxS	0	0
PFOS	0,01	0,01
PFDS	0,01	0,01

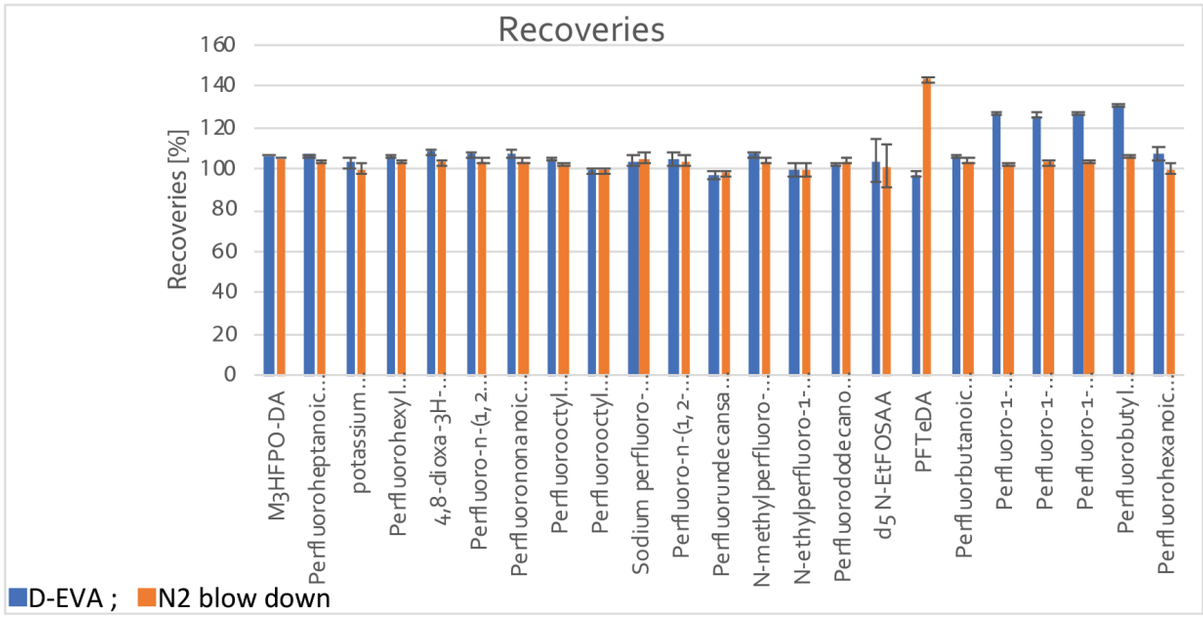

Fig. 2: Comparison of recovery rates

content in the eluate is sometimes impossible to avoid, which represents a further challenge for concentration. However, even samples with a water content of up to 25 % can be gently dried using the D-EVA.

In the experiment, 8 samples were spiked with surrogate standard solution, with a concentration in the middle calibration range, then mixed with different amounts of water and made up to 8 mL with methanol; all samples were evaporated with the D-EVA programme "Methanol". Any sample that is almost dry is considered finished. When all samples are almost dry, they are dissolved in methanol/water 96:4 % (v/v), spiked with internal standard and analysed by LC-MS/MS.

Due to the high sample throughput of the Freestyle-XANA, another main objective of the D-EVA application is to evaporate faster than the methodology recommended by US-EPA 537.1, without risking loss of analytes, while achieving desired signal intensity without any personnel supervision. In the respective rotors, 23 samples in 15 mL or 10 samples in 50 mL Falcontubes™ can be used in parallel. The fast programme evaporates the eluates to dryness within 11 minutes and the longer program, which is selected especially for water-contaminated samples, within 77 min. Thus, the two devices can be ideally combined in the laboratory and one can realise a workflow for a high sample-throughput.

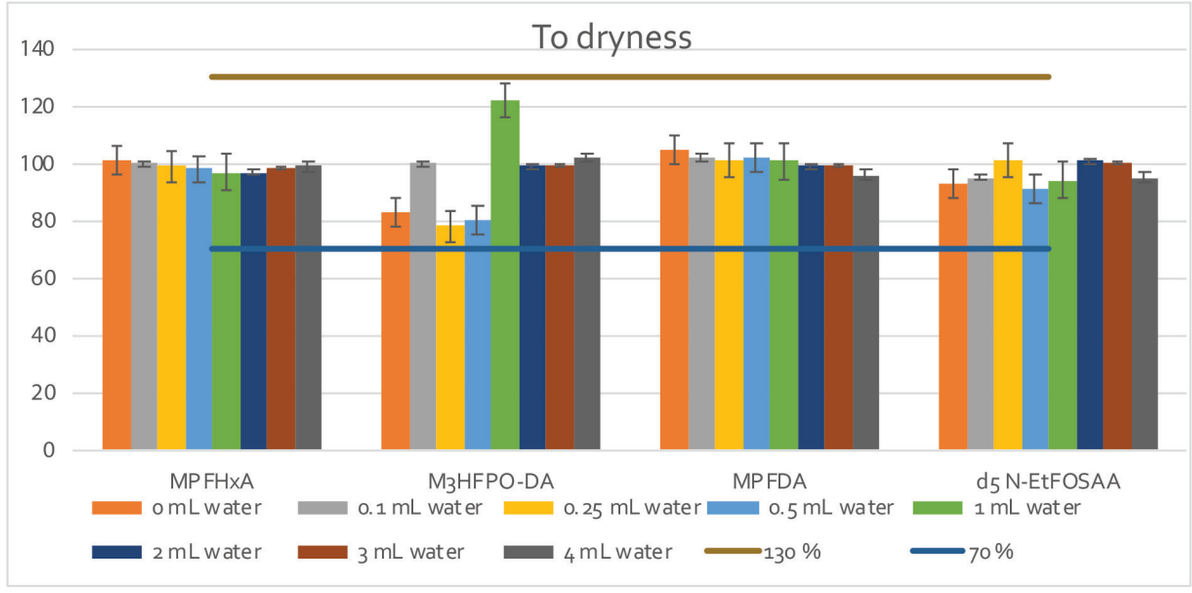

Fig. 3: Comparison of recoveries with regards to residual water

Conclusion

The analytical objectives of the applied method were met with good recoveries and very low standard deviations without cross-contamination, due to reliable and robust automation. Similarly, the analytical objectives of the DIN method were also met with high selectivity, trueness and precision. The use of the automated D-EVA vacuum centrifuge, which allows unattended parallel concentration of the collected PFAS eluate, enables gentle concentration, which achieves particularly high robustness.

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