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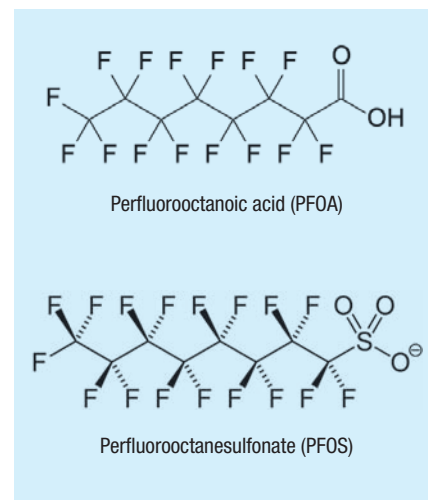
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Intelligent Automated Sample Preparation

Fast, accurate, and highly sensitive LC/MS determination of PFCs

The determination of Perfluorinated Compounds (PFCs) in water following the recognized international standard method (ISO/DIS 25101) is performed based on solid phase Extraction combined with HPLC-MS/MS determination. Application chemists have recently shown that if sample preparation and PFC determination is fully automated; the analysis can be performed more productively – and with significantly improved performance.

PFCs are creations of organic synthesis, developed in the R&D laboratory, produced in significant amounts and ubiquitous in the environment. PFC surfactants are synthesized from carboxylic acids or sulfonic acids with chain lengths of four to ten carbon atoms by substituting hydrogen atoms with fluorine atoms. Strictly speaking, PFCs can be divided into two groups: Perfluorinated alkylsulfonates (PFAS), among which perfluorooctanesulfonate (PFOS) is the most widely known compound, and perfluorinated carboxylic acids (PFCA), whose most famous representative is perfluorooctanoic acid (PFOA). Experts are of the opinion that PFCs only have limited toxicity. As to long term effects, there is no consensus even though PFCs have been reported as having cancer promoting properties and though the USEPA considers PFOA a “probable human carcinogen”. In fact, mainly the effects of PFOA and PFOS have been investigated while those of other



PFCs have been less extensively studied. The German Commission for Drinking Water lists an upper concentration limit of 1 µg/L.



The SPE-LC/MS/MS System used for this work. The dual rail MPS enables additional automation of liquid handling steps performed during sample preparation.

Developing an automated SPE-HPLC-MS/MS method

The automated method presented in this work is based on the current ISO standard method: "ISO 25101:2009 Water quality – Determination of perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) – Method for unfiltered samples using solid phase extraction and liquid chromatography/mass spectrometry". While the ISO method focuses on PFOS and PFOA, our goal for the project reported here was to develop a simple and rugged automated SPE-HPLC-MS/MS method for the determination of a wider list of PFCs in water and sludge. Automating the SPE process eliminates extensive and tedious manual sample preparation steps that are known sources of errors, for example when cartridges run dry or when sample matrix restricts the flow of liquids through the cartridge.

The benefits of automation are manifold: Higher recovery, improved reproducibility, higher sample throughput, increased flexibility and reduced exposure of laboratory staff to potentially hazardous solvents. Automated SPE can be performed using two different set-ups: The SPE system can be coupled directly to the HPLC-MS/MS instrument, enabling direct sample introduction of the extracts and fully automated operation from SPE to LC-MS/MS. Alternatively, the SPE system can be operated as a sample preparation workstation separate from the analysis instruments. The WorkStation set-up provides the flexibility of choosing between different techniques or different instruments for the sample analysis in order to meet individual requirements.



Instrumentation and Methods

River water and waste water samples that had been spiked with PFCs at different concentration levels were analyzed. Method development as well as the ensuing analysis was performed using an LC-MS/MS system from Agilent Technologies (1200 Series LC and 6400 Series Triple Quad LC/MS) combined with a GERSTEL SPE system. Two SPE systems were used, one based on the single rail MultiPurpose Sampler and one based on the dual rail MPS PrepStation, which offers additional liquid handling capabilities. The following compounds were determined using automated SPE-HPLC-MS/MS:

- Perfluorodecanoic acid (PFDA)
- Perfluorononanoic acid (PFNA)
- Perfluorooctanoic acid (PFOA)
- Perfluorooctanesulfonate (PFOS)
- Perfluoroheptanoic acid (PFHpA)
- Perfluorohexanoic acid (PFHxA)
- Perfluoropentanoic acid (PFPeA)

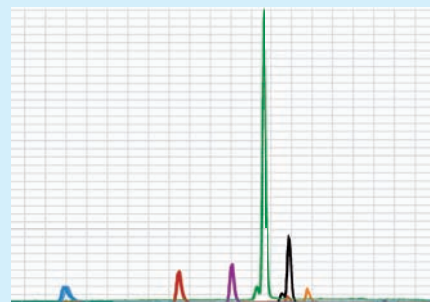
Isotopically labeled standards are used for quantitation in the ISO 25101 standard. In this work, perfluorobutanoic acid was used since this compound was not found in the samples.

HPLC Parameters

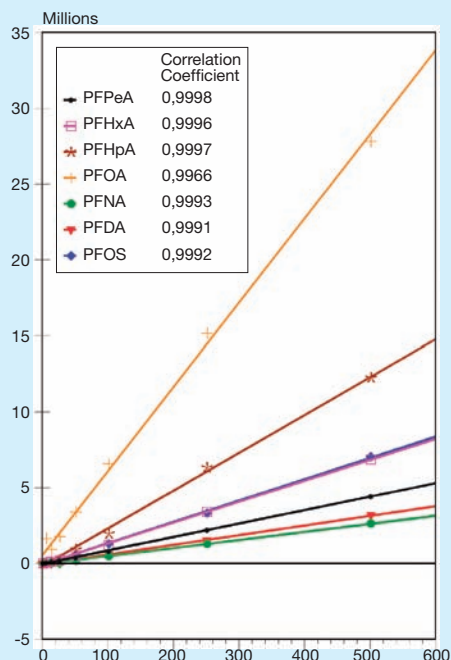
HPLC System: Agilent 1200 SL
 HPLC Column: Maisch Reprisil C18HD, 50 x 2.1 mm, 3 µm
 Flow: 0.3 mL/min
 Eluent: Ammonium acetate/
 Methanol (MeOH)
 Gradient: 0 min 20 % MeOH
 10 min 100 % MeOH
 14 min 100 % MeOH
 15 min 20 % MeOH
 Analysis time: 25 min
 Injection volume: 2 µL

MS Parameters

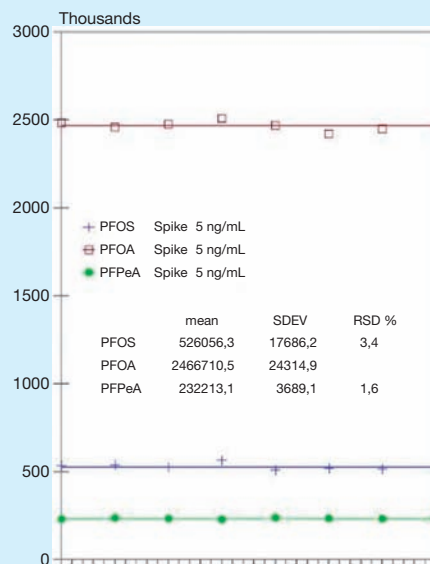
MSD: Agilent 6410 Triple
 Quadrupole
 Ionization mode: ESI neg.
 N₂ Temperature: 350 °C
 N₂ Flow: 10 L/min
 MS Mode: MRM (multiple
 reaction monitoring)
 Mass transfers: PFDA 513->469 m/z
 PFNA 463->419 m/z
 PFOA 413->369 m/z
 PFOS 499->99 m/z
 PFHpA 363->319 m/z
 PFHxA 313->269 m/z
 PFPeA 263->219 m/z



Overlay MRM traces of eight different sample preparations of a waste water sample spiked with 0.5 ng/mL. Excellent reproducibility is obtained for all compounds: PFPeA (blue), PFHxA (brown), PFHpA (purple), PFOA (green), PFNA (red), PFOS (black), PFDA (yellow)



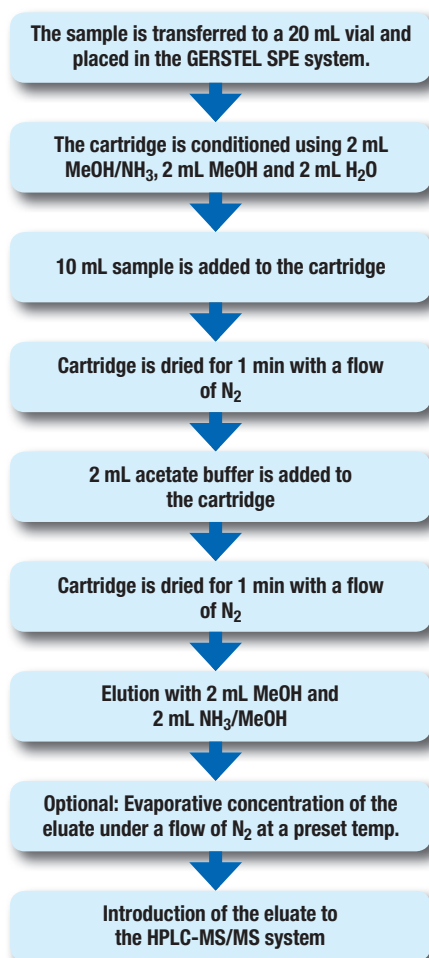
PFC calibration curves covering the range from 5 to 500 ng/mL based on external standard calibration.



Reproducibility of the method for PFOS, PFOA, PFPeA.



Just like the members of the infamous „dirty dozen“, i.e. the persistent organic pollutants (POPs), PFCs are ubiquitous. Mainly through industrial waste and waste water as well as through sludge, they end up in the environment. Without exaggeration, it is safe to say that PFCs are found in surface-, run-off- and ground water across planet Earth. They subsequently accumulate in the food chain, found in the livers of polar bears as well as in human blood.



This diagram shows the individual steps in the SPE process as performed by the MPS- or MPS PrepStation based SPE systems. Standard format cartridges of the type Oasis WAX 150 mg 6cc were used.

SPE Method

The GERSTEL MPS and GERSTEL PrepStation (Dual rail MPS) are both multi-functional autosamplers and sample preparation robots for GC/MS and LC/MS.

In addition to automated SPE using standard cartridge formats, practically all standard sample preparation techniques used for LC/MS and GC/MS can be performed automatically. This provides the user with significant added value: Method development tasks can be performed in a highly flexible manner and routine analysis chores handled efficiently and productively with minimal intervention. Among other benefits, the SPE systems are controlled through the MAESTRO software or integrated with the Agilent ChemStation and MassHunter. Just one sequence table is required to operate the entire system from SPE through liquid sample prep and sample introduction to GC/MS or LC/MS analysis. Everything is performed using just a few mouse-clicks. The PrepAhead function enables the system to plan ahead delivering time-optimized sample preparation. Samples are prepared just-in-time for introduction exactly when the LC/MS system becomes ready after the previous run, ensuring that the LC/MS is utilized to its fullest capacity. In this work, the sample preparation steps were completed in around 25 minutes.

Results and Discussion

The automated SPE-LC-MS/MS method presented in this work resulted in excellent calibration, sensitivity, recovery and reproducibility results. System performance was productive, reliable and rugged. Automated SPE is performed by the MPS or MPS PrepStation using commercially available standard volume cartridges. This means that existing manually performed SPE methods can easily be transferred to the automated system as is shown in the case of the ISO 25101 method. For every sample, a new cartridge is used eliminating the risk of cross contamination. Thanks to the PrepAhead function, LC/MS analysis of the current sample and SPE of the next sample in the sequence table are performed simultaneously, ensuring maximum productivity. For the PFC method, sample preparation took 25 minutes to complete. Apart from the time required to prepare the first sample for introduction, the LC/MS system is never slowed down by the sample preparation. Calibration based on samples spiked with 5 - 500 ng/mL of PFCs produced excellent linearity and reproducibility. Relative standard deviations were between 1 and 3 % depending on the compound. Following 2.5-fold enrichment of the SPE eluate, limits of quantitation were 0.5 ng/mL. Automated enrichment/concentration of the SPE eluate up to a factor 100 is possible and this would lead to a further significant reduction in the limit of quantitation.

Weighing Option

Automated weighing of liquid additions

An Automated Weighing Option is available for the GERSTEL MultiPurpose Sampler (MPS) based on a Sartorius ME laboratory balance. Standard autosampler vials are placed in the balance by the MPS and liquid samples, standards or diluents added, weighed and registered separately. For each sample, multiple liquid additions can be defined by mouse-click in the MAESTRO Software. Results are automatically transferred to pre-defined Microsoft Excel tables for convenient processing. The MPS Weighing Option simplifies the laboratory workflow and reduces the risk of operator error for improved convenience and productivity.



Conclusion and outlook

The automated SPE-LC/MS/MS method for determination of PFCs in water and sludge proved its worth in all aspects. The SPE process is performed using positive displacement pumping of sample and eluent, reducing matrix and restriction variation effects in the SPE cartridge in a simple and efficient manner. The automated method produces results of significantly higher quality than the ISO 25101 method in terms of both sensitivity and reproducibility, especially when analyzing difficult samples such as waste water.

The results of our work indicate that the automated method described in this work would be highly suitable for the determination of PFCs in other matrices such as urine and blood.