APPNOTE

GERSTEL
MAKING LABS WORK

GERSTEL AppNote 247

Determination of PFAS in Food of Animal Origin using online SPE Cleanup and LC-MS/MS

Claudia Sauer[1], Christin Pleger[1], Thomas Frenzel[1], Thomas Simat[2], Thomas Brandsch[3], and Oliver Lerch[3]

[1]*Landesuntersuchungsanstalt für das Gesundheits- und Veterinärwesen Sachsen, Reichenbachstraße 71/73, 01217 Dresden, Germany*
[2]*Technische Universität Dresden, Bergstr. 66, 01069 Dresden, Germany*
[3]*Gerstel GmbH & Co.KG, Eberhard-Gerstel-Platz 1, 45473 Mülheim an der Ruhr, Germany*

Keywords
Food analysis, LC-MS/MS, online-SPE, SPE^{xos}, PFAS

Abstract
In the work presented here, perfluorinated compounds were extracted from different food types (egg, fish and meat) using a QuEChERS-like approach followed by online-SPE cleanup and LC-MS/MS determination. In addition to validating the analysis method in accordance with the EU Guidance Document [1], matrix-based calibrations were compared to solvent-based calibrations to illustrate the matrix effects and demonstrate the cleanup efficiency. Online-SPE enables the injection of large amounts of sample extract, helping to reach the required quantification limits of 0.01 to 0.05 µg/kg.

Introduction
Per- and polyfluoroalkyl substances (PFAS) are a family of highly fluorinated anthropogenic chemicals with special physicochemical properties that make them oil and water repellant as well as heat resistant. This makes PFAS suited for many household and industrial applications like nonstick cookware, food packaging, carpeting, cleaning products, and firefighting foams. The unique chemical properties make them useful, but also difficult to break down. The lack of environmental degradation in combination with good solubility in water leads to a global distribution. PFAS are found not only in the environment, but also in food and animal feed, in humans, and in wildlife.

PFAS are toxic, and even acute exposure could have detrimental health effects. Authorities worldwide are regulating their use and emissions into the environment. In addition, food and drinking water must be monitored for their presence.

In a separate application note [2] the potential of online-SPE for the determination of PFAS in water was demonstrated. The same instrument configuration can be used to perform cleanup of extracts of solid materials like food, prior to LC-MS analysis. Unlike traditional SPE, online-SPE relies on smaller cartridges inserted into the eluent flow path that can be eluted directly and quantitatively onto the HPLC column. Using this technique, the efficiency of SPE is combined with the simplicity of direct injection. For the work reported here, an online-SPE system (GERSTEL SPE^{xos}, figure 1) was used that performs automated cartridge exchange as well as automated rinsing of the entire sample flow path between injections, ensuring that sample to sample carry over is reduced to an absolute minimum. All steps of a typical SPE workflow are performed automatically including conditioning, loading, washing, and eluting the cartridge. Following the elution step, the cartridge is removed from the HPLC mobile phase flow path, freeing the system to prepare the next sample during the ongoing LC-MS/MS analysis. The result is a fully automated sample preparation workflow that doesn't add to the overall analysis time once the first sample has been prepared and injected into the HPLC.

GERSTEL AppNote 247

Experimental

Materials and Solvents

For the extract cleanup, GERSTEL SPE^{xos} online SPE cartridges (Polymer WAX, GERSTEL 018804-023-00) were used. All solvents used were LC-MS grade.

Preparation of Samples and Calibration Standards

All standards were purchased as solutions from Wellington Laboratories (distributed by Campro Scientific, Berlin, Germany). Mixtures of isotopically labelled PFAS were used as internal standards for extraction and injection. All substances and their abbreviations are listed in Table 1.

Table 1: List of substances. Internal standards for extraction were used for the quantification of target analytes and internal standards for injection were used to determine the recovery rate. Analytes for which no isotopically labelled compounds are available were quantified using the internal standard most similar in structure and retention time.

Substance	Abbreviation	Molecular Formula	CAS No	Internal Standard for Extraction	Internal Standard for Injection
Perfluorobutanoic acid	PFBA	$C_4HO_2F_7$	375-22-4	$^{13}C_4$-PFBA	$^{13}C_3$-PFBA
Perfluoropentanoic acid	PFPeA	$C_5HO_2F_9$	2706-90-3	$^{13}C_5$-PFPeA	
Perfluorohexanoic acid	PFHxA	$C_6HO_2F_{11}$	307-24-4	$^{13}C_5$-PFHxA	
Perfluoroheptanoic acid	PFHpA	$C_7HO_2F_{13}$	375-85-9	$^{13}C_4$-PFHpA	$^{13}C_2$-PFOA
Perfluorooctanoic acid	PFOA	$C_8HO_2F_{15}$	335-67-1	$^{13}C_8$-PFOA	
Perfluorononanoic acid	PFNA	$C_9HO_2F_{17}$	375-95-1	$^{13}C_9$-PFNA	
Perfluorodecanoic acid	PFDA	$C_{10}HO_2F_{19}$	335-76-2	$^{13}C_6$-PFDA	$^{13}C_2$-PFDA
Perfluoroundecanoic acid	PFUnDA	$C_{11}HO_2F_{21}$	2058-94-8	$^{13}C_7$-PFUnDA	
Perfluorododecanoic acid	PFDoDA	$C_{12}HO_2F_{23}$	206-203-2	$^{13}C_2$-PFDoDA	
Perfluorotridecanoic acid	PFTrDA	$C_{13}HO_2F_{25}$	72629-94-8		
Perfluorotetradecanoic acid	PFTeDA	$C_{14}HO_2F_{27}$	376-06-7	$^{13}C_2$-PFTeDA	
Perfluorohexadecanoic acid	PFHxDA	$C_{16}HO_2F_{31}$	67905-19-5		
Perfluorooctadecanoic acid	PFODA	$C_{18}HO_2F_{35}$	16517-11-6		
Perfluorobutanesulfonic acid	PFBS	$C_4HO_3F_9S$	375-73-5	$^{13}C_3$-PFBS	
Perfluoropentanesulfonic acid	PFPeS	$C_5HO_3F_{11}S$	630402-22-1		
Perfluorohexanesulfonic acid	PFHxS	$C_6HO_3F_{13}S$	355-46-4	$^{13}C_3$-PFHxS	
Perfluoroheptanesulfonic acid	PFHpS	$C_7HO_3F_{15}S$	357-92-8		
Perfluorooctanesulfonic acid	PFOS	$C_8HO_3F_{17}S$	1763-23-1	$^{13}C_8$-PFOS	$^{13}C_4$-PFOS
Perfluorononanesulfonic acid	PFNS	$C_9HO_3F_{19}S$	98789-57-2		
Perfluorodecanesulfonic acid	PFDS	$C_{10}HO_3F_{21}S$	335-77-3		
Perfluorododecanesulfonic acid	PFDoS	$C_{12}HO_3F_{25}S$	79780-39-5		
Perfluorooctanesulfonamide	PFOSA	$C_8H_2O_2F_{17}NS$	754-91-6	$^{13}C_8$-PFOSA	
N-Ethyl-perfluorooctanesulfonamide	N-EtFOSA	$C_{10}H_6O_2F_{17}NS$	4151-50-2	$^{2}H_5$-N-EtFOSA	
8:2 Fluorotelomerphosphate diester	8:2 diPAP	$C_{20}H_9O_4F_{34}P$	678-41-1	$^{13}C_4$-8:2 diPAP	

Food samples (egg, meat, and fish) were processed in the following way: A 5 g sample, fortified with internal standard for extraction, was extracted twice with acetonitrile under alkaline conditions. After phase separation with sodium chloride, the organic phase was acidified with formic acid and frozen overnight. A first cleanup with dispersive SPE using $MgSO_4$ and Envi-Carb was followed by evaporative concentration to 0.3 mL, and after addition of internal standards for injection, brought to a final volume of 1 mL. Calibration in the range of 0.025 - 5 ng/mL (internal standards at 1 ng/mL) corresponds to 0.005 - 1 µg/kg food. The injection volume to online-SPE was 25 µL and for comparison, a direct injection of 2 µL to the LC-MS was performed.

GERSTEL AppNote 247

Instrumentation

The automated system consists of a MultiPurpose Sampler (MPS robotic, GERSTEL) and an online-SPE System (SPE^{xos}, GERSTEL) coupled to an LC-MS/MS system (1290 Infinity II Pump and LCMS 6495C, Agilent Technologies, Waldbronn, Germany). SPE elution is performed using 0.25% ammonia in methanol delivered from an additional isocratic HPLC pump (Infinity II 1260 Iso Pump, Agilent Technologies). The eluate is merged with the starting level buffer of the binary analytical pump in a valve fitted with a special T-rotor inside the SPE^{xos} system. As analytical column for the online-SPE-LC-MS/MS process, a Poroshell 120 EC-C18, 3 x 100 mm, 2.7 µm (Agilent Technologies) was used, and for direct injection a Zorbax Eclipse Plus C18 2.1 x 50 mm, 1.8 µm. Between the binary pump and MPS, a delay column (Poroshell 120 EC-C18, 4.6 x 50 mm, 2.7 µm, Agilent Technologies) was installed. Injection was performed with a 50 µL syringe into the injection valve on the MPS, fitted with a 100 µL or 2 µL stainless steel sample loop.

Figure 1: Online SPE system GERSTEL SPE^{xos}.

Analysis Conditions

The online-SPE workflow consisted of initially conditioning the cartridge, first using 0.25% ammonia in methanol, then methanol, and finally water. After injection of the sample into the loop, it was loaded onto the cartridge using water, and the cartridge was then washed. These steps were all performed by the High-Pressure Dispenser (HPD) unit of the SPExos (see figure 2). In this configuration the direct injection can occur through a second injection valve equipped with a 2 µL sample loop. One method with, and one without cartridge wash were used to compare the extract cleanup efficiency.

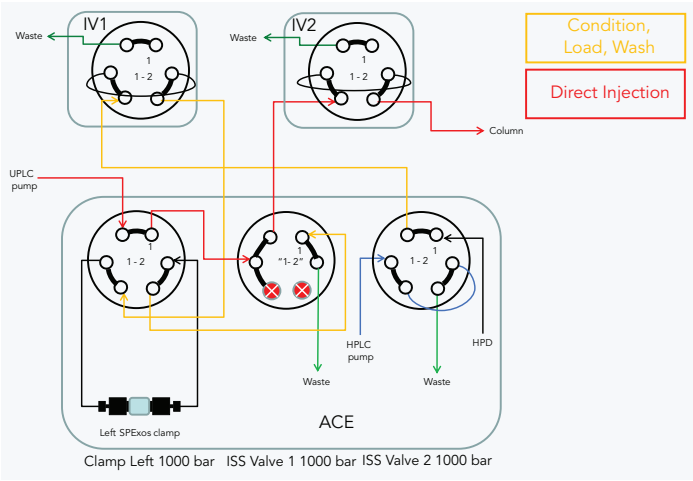

Figure 2: Flow path during conditioning, load, and wash of the cartridge. The same valve positions allow direct injection to LC/MS through injection valve IV2.

The isocratic pump elutes the cartridge with 0.25% ammonia in methanol and the binary pump delivers 0.1% formic acid in water, merged in the T-rotor valve of the SPE^{xos} (see figure 3). After the elution phase is completed, chromatography starts, and the binary pump delivers a gradient flow of 0.6 mL/min employing water with 0.1% formic acid and methanol with 0.25% ammonia and 0.05% formic acid. During this time the SPE^{xos} system can begin preparing the next sample (PrepAhead Mode).

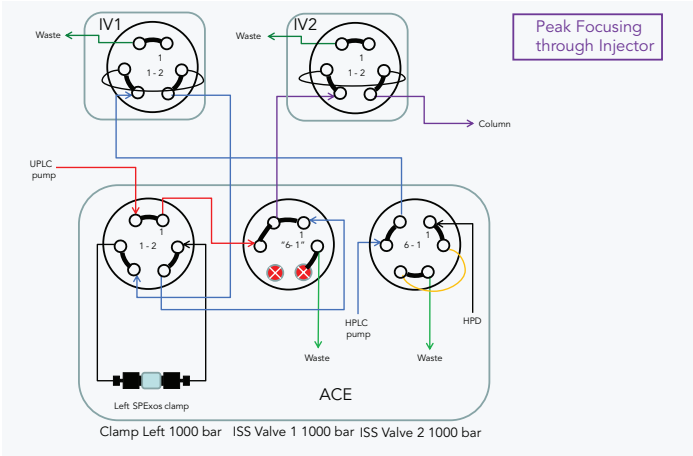

Figure 3: Flow path during elution of the cartridge with peak focusing on the LC column.

GERSTEL AppNote 247

Analysis Conditions LC

Pump	isocratic	
Mobile phase	0.25% NH_3 in methanol	
Flow rate	Time (min)	Flow (mL/min)
	0	0.1
	4.0	0.3
	5.0	0.0
	5.5	0.0
	6.0	0.5
	6.5	0.5
	7.0	0.0

Pump	binary		
Mobile phase	A – 0.1% formic acid in water		
	B – 0.25% NH_3 and 0.05% formic acid in methanol		
LC gradient	Time (min)	Flow (mL/min)	% B
	0	0.5	0
	4.0	0.3	0
	5.0	0.6	70
	14	0.6	100
	15	0.6	100
	16	0.4	0

Analysis conditions MS

Operation	dynamic multiple reaction monitoring mode (dMRM)
Gas temperature	250 °C
Gas flow (N_2):	11 L/min
Nebulizer pressure:	25 psi
Sheath gas flow (N_2):	11 L/min
Sheath gas temperature:	375 °C
Capillary voltage:	3000 V

For each target compound and isotope labeled internal standard, two MRM transitions were chosen, one quantifier and one qualifier (except PFBA und PFPeA, for which only one transition has sufficient intensity).

Results and Discussion

Usually in online-SPE, elution is performed using a gradient delivered by the analytical pump. However, the WAX cartridges are eluted with ammonia in methanol, which cannot be transferred directly to the HPLC column. For this reason, an extra (isocratic) HPLC pump is used to elute the cartridge, and the eluate is subsequently merged with the starting level buffer of the binary analytical mobile phase. It all takes place in the SPE^{xos} system using a valve fitted with a special rotor. During this stage the analytes reach the analytical column in an eluate mixture with a methanol content of 17%, which is then increased to 50%. The short chain PFAS begin to migrate on the column, but the longer chain PFAS are trapped at the beginning of the column. Switching the valve after 5 min ends the elution step and starts the gradient chromatography, during which the methanol content is increased rapidly to 70%, leading to focusing of first eluting peaks, while the later eluting peaks are separated in the second gradient stage.

Acidic compounds are retained on the WAX cartridge by ionic interactions, which means that the cartridge can be washed with organic solvents, resulting in the elution of most low and medium polar matrix components. In this work we performed washing with 300 µL of a mixture of acetonitrile/acetone/formic acid (50/50/1, v/v/v), 300 µL 0.01% formic acid in methanol and 300 µL acetonitrile. Figure 4 shows total ion chromatograms with (orange) and without (blue) cartridge wash (from left to right: egg, fish, meat), demonstrating that the cleaning effect is considerable. Non-ionic analytes like PFOSA and N-EtFOSA are removed from the cartridge during the wash procedure and can be analyzed only without using cartridge wash.

GERSTEL AppNote 247

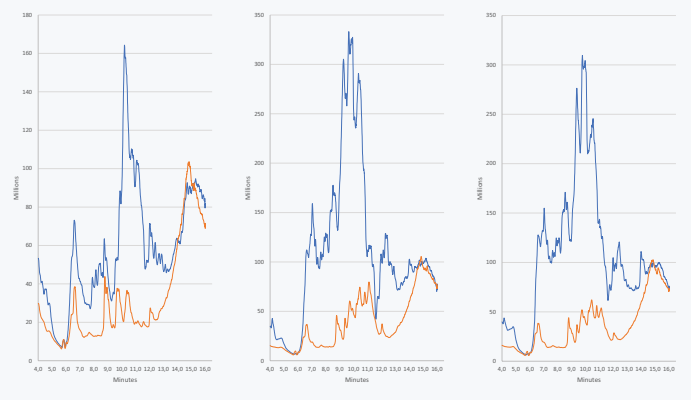

Figure 4: Comparison of total ion current chromatograms resulting from online SPE-LC-MS/MS analysis without and with cartridge wash for egg, fish and meat (from left to right).

Matrix Calibration

To investigate the matrix effects, samples of egg, fish and meat respectively were prepared without adding internal standards. Separate method calibrations based on the individual sample types were performed in an identical manner and compared to method calibrations obtained from standards in solvent in the range of 0.1-2 ng/mL (corresponding to 0.01-0.2 µg/kg in food, based on a 5 g sample). All solutions were analyzed by direct injection and by online-SPE, both with and without cartridge wash.

Using online-SPE for cleanup, it is possible to inject larger sample amounts without encountering matrix effects. We used an injection volume of 25 µL compared to 2 µL for direct injection. This would result in a signal increase by factor 12.5 and indeed the chromatograms obtained with online-SPE produced peaks with 10-13-fold larger areas compared with direct injection. The signal-to-noise ratios were similarly improved. The example in figure 5 shows mass traces for PFOS in egg resulting from direct injection of 2 µL (upper) and online-SPE injection of 25 µL extract (lower). As can be seen in the qualifier trace on the right, interferences are reduced in intensity with a retention time shift. Some analytes show considerably larger peak areas when cartridge wash has been performed, indicating that matrix induced ion suppression is being reduced. Figure 6 shows examples resulting from the analysis of egg samples, in which the difference is more or less pronounced (green versus orange lines; blue lines represent the areas for direct injection).

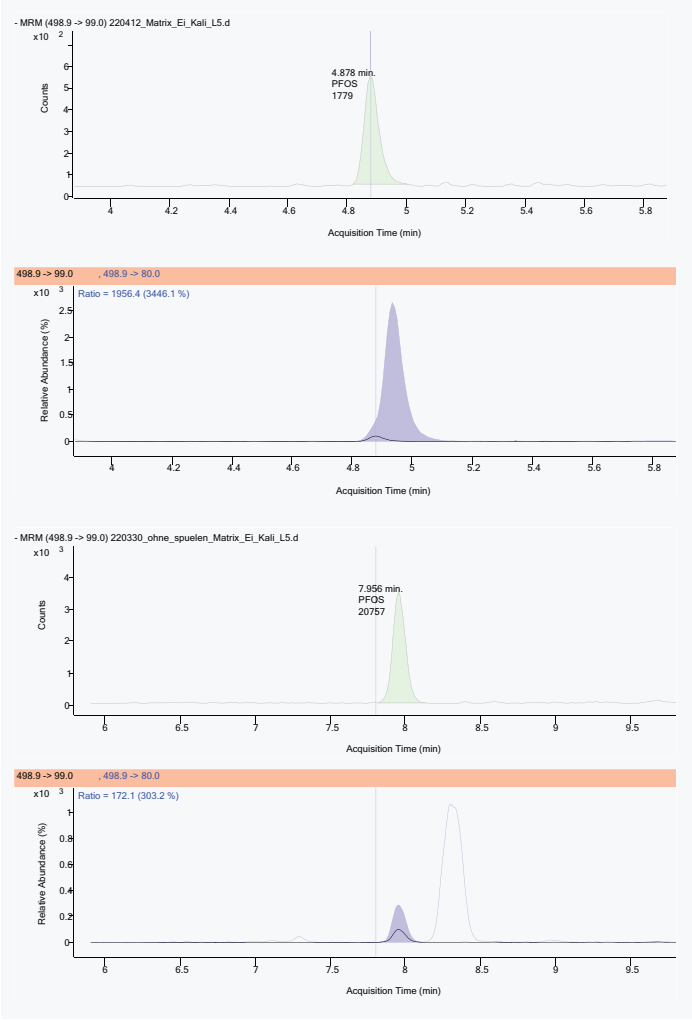

Figure 5: Sample chromatogram (quantifier and qualifier) for PFOS in egg from direct injection (upper) and online-SPE injection (lower).

GERSTEL AppNote 247

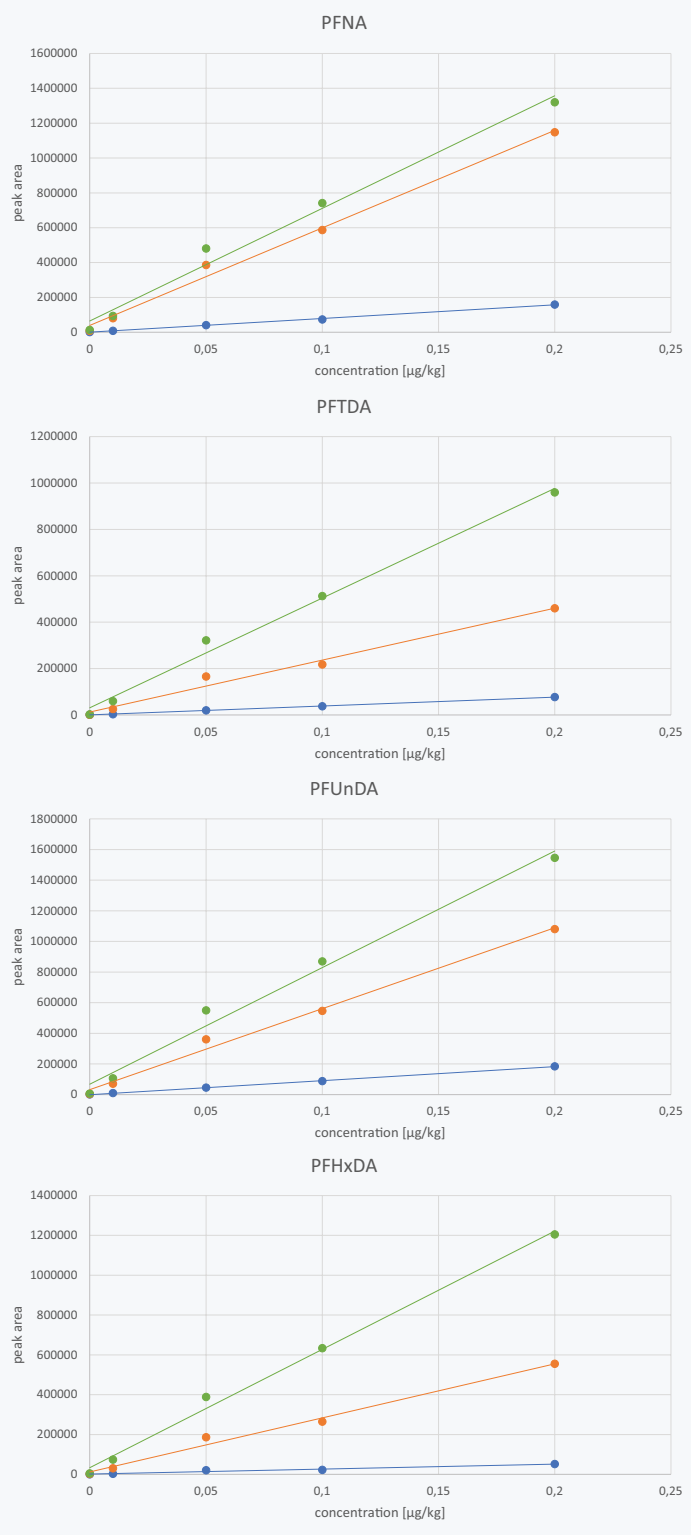

Figure 6: Comparison of peak area versus concentration (calibration) curves for selected compounds in egg obtained with online SPE and cartridge wash (green), online SPE without cartridge wash (orange) and direct injection (blue), respectively.

Matrix calibration is not always possible, and most often a solvent-based calibration is used, including a correction based on internal standards. If an isotopically labelled analyte is available, the relative response can be determined independently of possible matrix effects. But if the internal standard is only similar to the analyte, the relative responses can differ, and proper quantification is no longer possible. An example is PFHxDA, which is quantified using $^{13}C_2$-PFTeDA. Direct injection of 2 µL results in slight differences between the calibration curves resulting from solvent-based standards and those from matrix matched standards (figure 7, left). When injecting 25 µL into online-SPE without using cartridge wash, the calibration curves obtained for the different matrices all differ considerably from the calibration curve based on solvent standards. The more pronounced matrix effects are caused by the much larger amounts of sample injected (figure 7, middle). Washing the cartridge before elution reduces the matrix effects, and as a consequence the slopes of the calibration curves are similar (figure 7, right).

GERSTEL AppNote 247

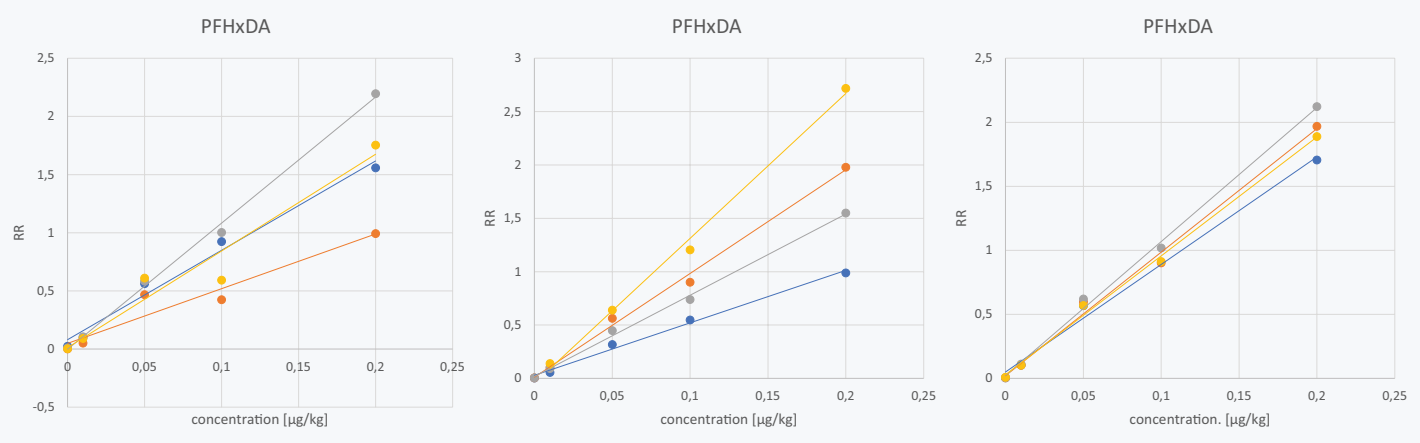

Figure 7: Comparison of relative response versus concentration for PFHxDA in solvent-based standard (blue), egg (orange), meat (grey) and fish (yellow) with direct injection (left), online SPE without cartridge wash (middle) and online SPE with cartridge wash (right).

Limits of Quantification

The final goal was to lower the limits of quantitation. According to the guidance documents, the limit of quantification represents the minimal concentration for which the quality criteria are met. Spiked samples at different concentration levels were analyzed in replicate to determine trueness and repeatability. Online-SPE enrichment and cleanup allows the injection of larger sample amounts and the reduction of matrix effects. Without cartridge wash, the recovery of some compounds is very low (e.g., $^{13}C_2$-PFTeDA) and therefore the signal increase was less than expected. Also, the mismatch with internal standards discussed earlier leads to overestimation of the analytes PFHxDA and PFODA and to large variations. However, the cartridge wash increases the recovery rate of these internal standards considerably, resulting in reduced variations (see table 2).

Table 2: Recovery rates of internal standards in online SPE measurement without and with column wash.

	Online SPE without column wash						Online SPE with column wash					
	Egg		Meat		Fish		Egg		Meat		Fish	
Internal Standard	Mean	RSD	Mean	RSD	Mean	RSD	Mean	RSD	Mean	RSD	Mean	RSD
$^{13}C_4$-PFBA	61.7%	15%	47.5%	15%	68.5%	6%	61.6%	15%	47.9%	15%	68.8%	8%
$^{13}C_5$-PFPeA	66.1%	13%	48.0%	16%	66.1%	9%	70.3%	13%	52.1%	15%	73.1%	12%
$^{13}C_5$-PFHxA	56.0%	10%	42.2%	14%	63.6%	8%	57.9%	13%	46.7%	14%	63.7%	7%
$^{13}C_4$-PFHpA	65.6%	9%	54.2%	11%	70.1%	7%	67.9%	10%	56.9%	13%	72.2%	5%
$^{13}C_8$-PFOA	69.1%	8%	60.8%	10%	73.3%	5%	70.2%	9%	62.7%	11%	75.1%	5%
$^{13}C_9$-PFNA	73.5%	7%	65.9%	8%	75.0%	6%	75.4%	7%	69.7%	11%	79.9%	5%
$^{13}C_6$-PFDA	70.4%	7%	67.0%	7%	73.7%	5%	71.3%	8%	69.3%	9%	76.2%	5%
$^{13}C_7$-PFUnDA	69.5%	8%	67.7%	6%	70.6%	6%	72.9%	9%	69.4%	11%	75.5%	7%
$^{13}C_2$-PFDoDA	66.6%	12%	68.9%	6%	46.6%	13%	76.8%	9%	72.6%	12%	77.8%	8%
$^{13}C_2$-PFTeDA	14.9%	18%	47.7%	11%	14.3%	19%	76.7%	12%	76.6%	10%	82.4%	10%
$^{13}C_3$-PFBS	68.2%	13%	68.7%	7%	77.6%	9%	71.9%	14%	74.9%	12%	75.1%	10%
$^{13}C_3$-PFHxS	75.2%	10%	74.8%	6%	79.3%	5%	78.1%	10%	78.1%	10%	78.7%	7%
$^{13}C_8$-PFOS	76.8%	8%	75.8%	5%	78.6%	7%	78.4%	12%	78.1%	9%	79.6%	5%
$^{13}C_8$-PFOSA	69.0%	11%	57.0%	24%	78.0%	10%	-	-	-	-	-	-
$^{2}H_5$-N-EtFOSA	21.0%	45%	16.2%	65%	2.4%	98%	-	-	-	-	-	-
$^{13}C_4$-8:2 diPAP	117.5%	10%	228.2%	12%	166.2%	20%	84.0%	16%	60.9%	9%	114.0%	29%

GERSTEL AppNote 247

Validation results with cartridge wash are shown in tables 3-5. Limits of quantification are 0.01 µg/kg for most compounds, lower than without cartridge wash and much lower compared to direct injection. Blank values introduced by the solvents used increase the limits of quantification for PFOA to 0.02 µg/kg and for PFPeA to 0.05 µg/kg. When analyzing egg, the sulfonic acids show larger matrix effects and therefore higher quantification limits of 0.05 µg/kg. The amides PFOSA and N-EtFOSA cannot be determined with cartridge wash. Quantification limits of 0.05 µg/kg and 0.5 µg/kg were achieved without cartridge wash, except for fish in which N-EtFOSA could not be quantified in the expected concentration range. Without cartridge wash, the blank values for PFBA were lower, allowing a quantification limit of 0.05 µg/kg. This compound shows only one MS transition and results therefore have to be verified on a different column (for example HILIC).

Table 3: Validation data for PFAS in egg.

	Spike level 0.01 µg/kg			Spike level 0.05 µg/kg			Spike level 0.5 µg/kg		
Substance	Mean conc. [µg/kg]	Recovery	RSD	Mean conc. [µg/kg]	Recovery	RSD	Mean conc. [µg/kg]	Recovery	RSD
PFPeA	0.012	117%	26.2%	0.054	107%	13.5%	0.520	104%	4.5%
PFHxA	0.011	106%	10.0%	0.053	106%	4.7%	0.496	99%	2.6%
PFHpA	0.010	98%	17.5%	0.053	107%	8.5%	0.495	99%	3.1%
PFOA	0.012	117%	7.3%	0.055	110%	5.3%	0.496	99%	5.1%
PFNA	0.010	102%	5.5%	0.053	106%	6.0%	0.484	97%	4.2%
PFDA	0.011	106%	4.8%	0.053	107%	6.2%	0.498	100%	2.9%
PFUnDA	0.011	114%	6.1%	0.054	107%	6.9%	0.505	101%	4.0%
PFDoDA	0.011	112%	5.6%	0.054	109%	7.2%	0.497	99%	4.0%
PFTrDA	0.010	100%	6.9%	0.050	101%	4.8%	0.481	96%	4.1%
PFTeDA	0.011	107%	7.1%	0.052	104%	6.2%	0.484	97%	3.3%
PFHxDA	0.010	97%	10.1%	0.047	94%	8.5%	0.432	86%	6.1%
PFODA	0.008	82%	10.8%	0.039	77%	12.2%	0.349	70%	10.0%
PFBS	0.011	107%	14.2%	0.051	101%	6.2%	0.435	87%	4.4%
PFPeS	0.012	123%	18.7%	0.048	96%	9.9%	0.457	91%	3.3%
PFHxS	n.d.	-	-	0.037	74%	12.1%	0.512	102%	6.3%
PFHpS	0.011	114%	40.7%	0.052	103%	12.5%	0.445	89%	5.2%
PFOS	0.013	126%	14.6%	0.052	104%	10.8%	0.468	94%	3.5%
PFNS	0.012	119%	17.5%	0.049	98%	16.1%	0.446	89%	6.5%
PFDS	0.010	97%	19.5%	0.052	103%	11.2%	0.459	92%	5.6%
PFDoS	0.010	101%	50.1%	0.054	107%	11.7%	0.448	90%	11.0%
8-2 diPAP	0.011	111%	11.4%	0.051	101%	7.2%	0.466	93%	4.2%
Without cartridge wash									
PFBA	0.019	191%	74.9%	0.057	114%	5.6%	0.508	102%	2.5%
PFOSA	0.011	114%	7.7%	0.054	107%	6.1%	0.494	99%	4.1%
N-EtFOSA	n.d.	-	-	0.073	146%	30.8%	0.595	119%	13.3%

GERSTEL AppNote 247

Table 4: Validation data for PFAS in meat.

	Spike level 0.01 µg/kg			Spike level 0.05 µg/kg			Spike level 0.5 µg/kg		
Substance	Mean conc. [µg/kg]	Recovery	RSD	Mean conc. [µg/kg]	Recovery	RSD	Mean conc. [µg/kg]	Recovery	RSD
PFPeA	0.014	143%	42.5%	0.056	112%	7.0%	0.519	104%	5.2%
PFHxA	0.011	108%	19.1%	0.055	109%	8.7%	0.496	99%	4.6%
PFHpA	0.011	108%	16.1%	0.051	103%	6.9%	0.483	97%	4.8%
PFOA	0.010	103%	21.7%	0.052	103%	7.6%	0.475	95%	5.1%
PFNA	0.011	109%	9.9%	0.053	106%	8.5%	0.472	94%	4.0%
PFDA	0.010	104%	11.0%	0.052	104%	8.9%	0.478	96%	3.8%
PFUnDA	0.011	113%	6.7%	0.054	108%	9.0%	0.506	101%	4.8%
PFDoDA	0.011	110%	6.5%	0.054	108%	7.8%	0.494	99%	4.9%
PFTrDA	0.011	107%	5.8%	0.053	106%	8.1%	0.487	97%	5.7%
PFTeDA	0.010	104%	4.2%	0.051	101%	8.9%	0.469	94%	4.5%
PFHxDA	0.009	93%	7.6%	0.046	92%	10.9%	0.423	85%	6.0%
PFODA	0.008	78%	9.3%	0.038	76%	13.4%	0.348	70%	7.2%
PFBS	0.011	110%	9.3%	0.048	96%	5.6%	0.425	85%	2.8%
PFPeS	0.011	106%	5.0%	0.048	95%	8.2%	0.444	89%	3.1%
PFHxS	0.011	111%	7.4%	0.052	103%	4.6%	0.461	92%	3.2%
PFHpS	0.010	99%	12.6%	0.048	96%	8.4%	0.458	92%	5.3%
PFOS	0.011	107%	21.4%	0.055	110%	10.1%	0.460	92%	3.6%
PFNS	0.012	124%	22.0%	0.053	105%	8.5%	0.459	92%	4.2%
PFDS	0.010	101%	11.8%	0.052	103%	7.1%	0.451	90%	6.8%
PFDoS	0.011	114%	19.4%	0.052	104%	14.2%	0.451	90%	7.9%
8-2 diPAP	0.011	109%	8.8%	0.050	100%	11.2%	0.453	91%	5.2%
Without cartridge wash									
PFBA	0.011	110%	19.4%	0.054	108%	15.5%	0.497	99%	3.7%
PFOSA	0.011	108%	7.0%	0.053	105%	9.0%	0.485	97%	4.4%
N-EtFOSA	0.002	20%	153.9%	0.032	63%	102.8%	0.555	111%	11.5%

Table 5: Validation data for PFAS in fish.

	Spike level 0.01 µg/kg			Spike level 0.05 µg/kg			Spike level 0.5 µg/kg		
Substance	Mean conc. [µg/kg]	Recovery	RSD	Mean conc. [µg/kg]	Recovery	RSD	Mean conc. [µg/kg]	Recovery	RSD
PFPeA	0.010	96%	18.0%	0.052	105%	5.8%	0.515	103%	3.4%
PFHxA	0.012	122%	15.3%	0.053	106%	5.3%	0.503	101%	1.8%
PFHpA	0.010	103%	21.8%	0.052	103%	9.9%	0.497	99%	0.3%
PFOA	0.011	108%	12.6%	0.052	104%	5.1%	0.492	98%	1.5%
PFNA	0.011	114%	3.8%	0.053	105%	2.2%	0.488	98%	1.3%
PFDA	0.010	105%	7.4%	0.053	105%	6.2%	0.495	99%	1.5%
PFUnDA	0.011	113%	8.0%	0.055	109%	5.1%	0.516	103%	2.0%
PFDoDA	0.011	113%	7.1%	0.054	108%	5.9%	0.512	102%	1.4%
PFTrDA	0.011	108%	4.0%	0.052	104%	5.5%	0.494	99%	2.6%
PFTeDA	0.011	106%	5.5%	0.051	103%	5.5%	0.484	97%	2.4%
PFHxDA	0.011	113%	8.8%	0.054	108%	7.4%	0.513	103%	2.2%
PFODA	0.011	107%	12.7%	0.050	101%	9.8%	0.491	98%	3.0%
PFBS	0.011	115%	5.0%	0.049	99%	5.9%	0.433	87%	2.4%
PFPeS	0.010	104%	4.6%	0.046	92%	4.4%	0.459	92%	4.7%
PFHxS	0.011	108%	6.0%	0.054	108%	6.2%	0.471	94%	3.7%
PFHpS	0.010	99%	9.4%	0.046	93%	11.6%	0.468	94%	2.7%
PFOS	0.012	121%	16.9%	0.057	114%	14.0%	0.473	95%	2.9%
PFNS	0.012	119%	19.4%	0.050	101%	13.0%	0.478	96%	3.3%
PFDS	0.010	103%	13.2%	0.051	103%	11.5%	0.475	95%	3.9%
PFDoS	0.010	103%	22.3%	0.054	108%	14.3%	0.484	97%	6.9%
8-2 diPAP	0.011	107%	6.7%	0.051	101%	6.1%	0.474	95%	2.1%
Without cartridge wash									
PFBA	0.002	19%	661.9%	0.043	86%	22.2%	0.506	101%	2.4%
PFOSA	0.014	136%	24.1%	0.053	107%	9.3%	0.493	99%	1.9%
N-EtFOSA	n.d.	-	-	n.d.	-	-	n.d.	-	-

Conclusions

The online-SPE-LC-MS/MS system combined with the presented method enables automated cleanup of food extracts and determination of PFAS compounds in the ng/kg range. Due to the cleanup effect of online-SPE, the quantification limits compared to direct injection are much lower. The organic wash of the cartridges prior to elution effectively removes matrix interferences and improves the accuracy of the results. The method accuracy and trueness were demonstrated for different food types of animal origin (egg, meat, and fish). The main benefits compared to traditional SPE are simple sample handling, very low solvent consumption and excellent reproducibility.

References

[1] Guidance Document on Analytical Parameters for the Determination of Per- and Polyfluoroalkyl Substances (PFAS) in Food and Feed, Version 1.2, 11 May **2022**.

[2] T. Brandsch, O. Lerch, Gerstel AppNote 237: *Determination of PFAS in Water according to EU 2020/2184 and DIN 38407-42 using online-SPE-LC-MS/MS*, June **2022**.