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Review: Extraction and purification methods for PFAS analysis in food – Proposal for Automatization

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Goal

1 To present a summary on recent methodologies for the extraction and purification of PFAS in food

2 To propose an extraction and purification method using automated off-line SPE

Introduction

Perfluoroalkyl and polyfluoroalkyl substances (PFAS) [1] are a class of over 6000 manufactured compounds. These compounds have been used for over 80 years for numerous applications in industry and consumer products, like among others surfactants and coating materials. The widespread use of these compounds have led to food contamination through bioaccumulation, transfer from contact materials and exposure to environmental contamination[2]. The widespread occurrence has raised concern for the use of these chemicals, and has urged governmental bodies to assess the risk that these compounds oppose to human health[2].

The reason for the vast use of PFAS was due to their unique chemical structure providing for both hydrophobic and lipophilic properties as well as di-electricity, resistance to heat and chemical agents, and low surface energy[3]. The chemical structure of PFAS consists of a hydrophilic alkyl chain of varying length and branching degree, and a varying hydrophilic end group, most commonly carboxylic acid (fig. 1) or sulfonic acids (fig. 2) (i.e. PFCAs and PFSAs). Initial research has focused on PFSAs and PFCAs, but more recent studies also pay attention to their derivates like perfluoroalkane sulphonamides, fluorotelomer substances, perfluoroalkane sulfonamido substances and polyfluoroalkyl phosphate esters[4].

As their names imply PFCAs and PFSAs (together PFAAs) can be present in the anionic and neutral form (acidic form)[1]. Generally laboratories use the acidic or neutral nomenclature, yet many PFAAs are present in their dissociated anionic form. The dissociation state changes the chemical properties and should be considered when developing methodological strategies to extract these compounds from the matrix[5,6]. In addition, these properties also allow for specific purification approaches, like anion exchange based purifications[7].

In this study, recent and foundational analytical methods are summarized and discussed. Moreover, an automated purification step for the analysis of PFAS in food using the Promochrom's SPE-03 high volume automated SPE system (fig. 3) is proposed and evaluated.

Figure 1 Molecular structure of perfluorooctanoic acid (PFOA).

Figure 2 Molecular structure of perfluorooctane sulfonic acid (PFOS).

Methods: extraction

Several types of extraction methods were employed over the studies (table 1). The most frequently used methods were straightforward solid- liquid extraction (SLE), ion-pair extraction (IPE) and SLE after alkaline digestion. For straightforward SLE, methanol, acetonitril, water, or a combination of these solvents were used. For alkaline digestion, the same solvents were used, but prior to extraction, the sample matrix

was digested using 2 mM to 10 mM sodium hydroxide (NaOH) or pottasium hydroxide (KOH) solution. When IPE was applied, the extraction generally involved the use of methyl tert-butyl ether (MTBE) in combination with ion paring agents like tetrabutylammonium (TBA), tetrabutylammonium hydrogen sulfate (TBAHS), sodium carbonate (Na_2CO_3) and sodiumbicarbonate ($NaHCO_3$). In several cases a QuEChERS extraction was applied, while in fewer cases pressurized liquid extraction (PLE), liquid liquid extraction (LLE) and matrix solid phase dispersion (MSPD) were applied. The latter three approaches will not be further discussed as the other extraction methods (i.e. SLE and IPE) prevailed in numbers in the literature.

One trend that is observed in extraction techniques, is the use of additional chemical properties or reactions to extract the PFAS from animal tissue. For instance, by alkaline digestion, biological material like proteins, nucleic acids, carbohydrates, lipids, are hydrolized into an aqueous solution making the compounds more available for extraction[8]. This is necessary because PFAS are considered to be strongly incorporated in the portein structure due to its specific protein and lipid binding properties[9]. Therefore, an extraction with alkaline digestion is considered to improve accuracy and reliability for biological samples[10].

Extraction efficiency is also considered to improve when PFAS are present in their neutral (acidic) form. Zhou et al.[5] reported in 2019 higher recoveries when formic acid (FA) was added to the extraction solvents. The determined pH values were between 3.18 and 3.64 for the different experiments[5]. Many PFAA are considered to be strong acids and for PFOA predicted pKa values range from -0.5 to 3.8[11,12,13,14] and for other PFAAs estimates are even lower (pKa<1.6)[15]. Hence, the higher recoveries reported by Zhou et al.[5] at pHs between 3.18 and 3.64 might have been for other reasons than the dissociation state.

In IPE, a way to overcome polarity differences between the PFAS and the organic solvent (MTBE) is to introduce an oppositely charged ion. The two oppositely charged ions can form an ion-pair that diffuses more easily into the organic phase[16]. Although this IPE method has been widely applied, it was considered to have disadvantages, like co-extraction of matrix constituents (like lipids due to the use of MTBE) which can trouble instrumental analysis in the absence of a purification step[10]. In more recent studies, IPE was combined with alkaline digestion[17,18] and solid phase extraction (SPE)[18] purification to improve the purity of the extracts.

QuEChERS is considered accurate and highly productive at ultra trace levels[10]. Yet, for the analysis of PFAS in food, this method is not widely applied compared to the straighfoward SLE and IPE methods. Recently, a one step QuEChERS extraction and purification was found to be successful[5]. However, a widespread application of QuEChERS methods for the analysis PFAS in biological samples still hasn't been demonstrated.

SLE with a mixture of tetrahydrofuran (THF) and water was applied after sound theoretical considerations[19]. THF and water were chosen for their substantial difference in solvation capability being dispersion force, dipole-dipole force and hydrogen bonding (Hildebrand solubility parameters)[19]. Using the solvents separately, recoveries for several to all PFAS remained insufficient. A mixture of THF:water (75:25 v/v) was found to be optimal. As water content between samples vary, freeze drying of the samples was required. Recoveries were in the acceptable range of 70% - 120%[19].

As for solvents used, MTBE is non polar and well capable of coextracting lipids from biological samples. Therefore a further clean-up is desirable when using this solvent[10]. A combiantion of water and methanol is thought to be unsuitable for long chain PFAAs as these compounds are only slightly soluable in a mixture of water and methanol[10]. Acetonitrile and methanol generally resulted in sufficient recoveries like for when MTBE is used.

Several factors like ease of use, laboratory safety, available resources and common methods at laboratories will influence the method of choice for the extraction of PFAS. The THF/water approach is very promising in terms of recovery and precision, but involves dangerous chemicals and a well adjusted water content in samples. QuEChERS methods seem

Table 1A. A summary of employed extraction methods in scientific publications.

Reference	Sample type	Freeze dried	Sample intake	# PFAS	Extraction method	Agitation	Extraction solvent	Solvent switch	Recovery (incl. Purification)
2010 Ballesteros-Gomez et al.[19]	herring, pangasius, salmon and flounder,) meat (pork and chicken), whole-grain bread, spinach and carrot, fruits orange and apple, cheese (Gouda) and sunflower oil	yes	10 g	14	SLE	orbital shaker	THF/water (v/v 75:25)	yes/no	60% - 120%
2011 Kadar et al.[20]	breast milk	no	3 ml	14	LLE	vortex ultra-sonication	acetone	yes	38%-105% (250 pg/ml) 61% - 104% (750 pg/ml)
2012 Vestergren et al.[21]	baby food composite, fish composite, meat composite and vegetable composite	no	2.5 g	9	IPE	vortex	MTBE with ion pair (NaOH 0.4 M, Na_2CO_3/$NaHCO_3$ buffer 0.25 M (adjusted to pH 10), 0.5 M TBA)	no	49% - 67%
2012 Valsecchi et al.[10] (summary of 32 reported analysis)	fish, shellfish (muscle tissue and organs)				IPE n=7, SLE n=16, SLE alkaline n=8, QuEChERS n=1, PLE n=1, MSPD n=1	sonication n=8, shaking n=8,	MTBE (TBA) n=6, MTBE (NaOH, TBA) n =1, MTBE n=1, acetonitrile n=7, acetonitrile FA n=2, methanol:water NH4Ac n=1., methanol KOH/NaOH n =8, THF:water n=2, water n=1, hexane:acetone n=1, HCl:HNO3 n=1		generally good for PFCA and PFSA (60-120%)
2014 Perez et al.[22]	foodstuff classes (cereals, fish, fruit, milk, ready-to-eat foods, oil and meat)	no	2 g	21	SLE alkaline	orbital shaker	methanol 20 mM NaOH		70% - 120%
2015 Gebbink et al.[4]	(products of) dairy, meat, fats, pastry, fish, egg, cereal, vegetables (incl roots), fruit, potatoes, sugar and sweets, soft drinks	no	2.5-5 g	23	SLE LLE	mixing sonication	acetonitrile	no	1% 262%
2016 Zafeiraki et al.[23]	egg yolk	no	1 g	11	SLE alkaline	vortex	water (2 mM NaOH) + methanol (extraction) + HCl (neutralization)	yes	40% - 115%

Table 1B (continuation of table 1A) A summary of employed extraction methods in scientific publications.

Reference	Sample type	Freeze dried	Sample intake	# PFAS	Extraction method	Agitation	Extraction solvent	Solvent switch	Recovery (ncl. Purification)
2016, 2017 Navarro et al.[24,25]	waste, soil, earthworm, spinach, tomato and corn	yes	-	11	SLE	agitation ultrasonication	acetonitrile	-	48% - 104%
2017 Xiang et al.[7]	cereal, root vegetable, leafy vegetable and melon vegetable	yes	0.5 g	9	SLE	mixing sonication	twice acetonitrile/water v/v 9:1 and once acetonitrile	yes	70% - 114%
2017 Pignotti et al.[26]	sediment and fish tissue	sediment was dried	1 g	13	SLE SLE alkaline	soil - sonication fish - orbital shaker	soil- methanol, fish - methanol (10 mM NaOH) + methanol (extraction)	yes	
2017 Akerblom et al.[27]	fish tissue	yes	1 g	26	SLE		methanol	no	
2019 Wu et al.[28]	egg (homogenate)	no	1 g	27	SLE alkaline		acetonitrile/water v/v 80:20 (10 mM KOH)	yes	x̄ 47% - 118%, RSD 5% - 29%, accuracy 87% - 129%
2019 Sznajder-Katarzynska et al.[29]	milk, kefir, yoghurt, cottage cheese, sour cream, camembert and butter	no	10g or 10ml	10	QuEhERS	sonication mechanically	acetonitrile 1.5% FA (in addition of 1 g NaCl and 4 g MgSO$_4$)	no	x̄ 50% - 140%
2019 Sonne et al.[30]	meat from seal	no	5 g	15	SLE	Ultra-sonication	Acetonitrile	no	75% - 129%
2019 Fair et al.[17]	fish tissue incl skin	no	1 g	11	IPE	mechanically	water (0.25 M Na$_2$CO$_3$ and 0.5M TBASH (pH 10) + MTBE (extraction)	-	90% - 118%
2019 Bao et al.[18]	soil, tomato, cucumber, eggplant, pepper, Chinese cabbage, eggs	soil was dried	2 g	10	IPE		water (0.25 M Na$_2$CO$_3$ and 0.5M TBASH (pH 10) + MTBE (extraction)	yes	genreal statement 80% - 120%
2019 Y. Zhou et al.[5]	validated cucumber, lettuce, eggplant, tomato and leek - Analyzed pepper, zucchini, wax gourd, water spinach, long bean, rape and loofah	no	15 g	20	QuEhERS	Vortex	acetonitrile containing 1% FA - 1.5 g NaOAc and 6 g MgSO$_4$	no	55% - 119%

Table 2 A summary of employed purification methods in scientific publications

Reference	Solvent applied to purification	Method	Sorbents	Wash	Elute	Concentration
2010 Ballesteros-Gomez et al.[19]	THF:water (3.5:11.5 v/v)	SPE (non-retentive)	WAX + Envi-Carb	water (25mM acetate buffer), THF:acetonitrile (50:50 v/v)	methanol (0.1% NH$_4$OH)	dryness (vacuum)
2011 Kadar et al.[20]	acetone:water 0.1M FA (3:8 v/v)	SPE (non-retentive)	Oasis HLB + Envi-Carb	water (100 mM FA), methanol (100 mM FA)	methanol (1% NH$_4$OH)	dryness
2012 Vestergren et al.[21]	MTBE (3ml concentrated extract)	SPE non-retentive	Na$_2$SO$_4$, Florisil and Envi-Carb	MTBE	methanol:MTBE (30:70 v/v)	reduction (N$_2$)
2012 Valsecchi et al.[10] (summary of 32 reported analysis)		n.a. /filter n=8, SPE n=12, SPE non-retentive n=6, dSPE n=5, H2SO4 n=1	n.a. /filter n=8, WAX (+Envi-Carb) n=9, HLB n=2, dSPE Envi-Carb n=5, Strata X-AW n=1, Online SPE, silica gel, H$_2$SO$_4$			dryness (N$_2$) n=12, reduction (N$_2$) n=6, n.a. n=13
2014 Perez et al.[22]		TFC			water (FA pH 4.5)	-
2015 Gebbink et al.[4]	acetonitrile (1ml concentrated extract)	SPE	WAX	water (2% FA), water	methanol (neutral PFAS), methanol (1% NH$_4$OH) (anionic PFAS)	dryness (N$_2$)
2016 Zafeiraki et al.[23]	water:methanol (25:10 v/v)	SPE	WAX	water (25 mM NaOAc (pH4))	acetonitrile (2% NH$_4$OH)	dryness (N$_2$)
2016, 2017 Navarro et al.[24,25]	acetonitrile (5% acetic acid (AA))	SPE non-retentive	Envi-Carb	-	-	dryness (N$_2$)
2017 Xiang et al.[7]	water:acetonitrile (concentrated extract) (9:1 v/v)	SPE (non-retentive)	WAX + Envi-Carb	-	methanol (0.1% NH$_4$OH)	dryness (N$_2$)
2017 Pignotti et al.[26]	water:methanol 9:1	TFC			-	-
2017 Akerblom et al.[27]	methanol	dSPE	dSPE Envi-carb + glacial acetic acid		-	-
2019 Sznajder-Katarzynska et al.[29]	acetonitrile (1.5% FA)	dSPE, Freeze out, Filtration	ENV and MgSO$_4$, paper	-	-	dryness (vacuum)
2019 Sonne et al.[30]	acetonitril (2.5% FA)	SPE non-retentive	Envi-Carb	-	methanol	dryness (N$_2$)
2019 Fair et al.[17]	-	-	-		-	dryness (N$_2$)
2019 Bao et al.[18]	water:methanol (50:1 v/v)	SPE (non-retentive)	WAX + Envi-Carb (+ glacial acetic acid)	water (acetate buffer), methanol	methanol (2% NH$_3$)	reduction (N$_2$)
2019 Y. Zhou et al.[5]	acetonitrile (1% FA)	SPE non-retentive	90mg PSA, (80mg C$_{18}$) and 15mg MWCNTs	-	-	dryness (N$_2$)

promising as an allround extraction method, while SLE or IPE with alkaline digestion theoretically should result in the most accurate results as protein bound PFAS are released from the matrix. Due to the digestion step, both methods involve the use of multiple chemicals which makes these methods more elobrated compared to straightforward SLE[31].

Methods: purification

In many studies the supernatant of the extraction was reduced and diluted with either water, methanol, acetonitrile, acetone or a combination of these solvents. For purification of these extracts, several approaches were used (table 2). In mostly earlier researches, no purification was applied. However, nowadays, most publications report the use of a (optimized) purification step. Typically, SPE is being used for cleaning up the extract by either non-retentive SPE or by retentive SPE[32]. The latter is in this study referred to as SPE. Non-retentive SPE was also applied in a dispersive approach (dSPE)) or by turbulent flow chromatography (TFC) to remove interferences from the extract. In a single study freezing the aliquot was performed to remove lipids followed by paper filtration and in another sulfuric acid was used.

For IPE, in earlier researches purification was generally not performed in combination with IPE[10]. In one study, hexafluoroisopropanol was used for matrix precipitation while keeping the PFAS dissolved by its "fluorophilicity"[33]. In later research, IPE was also combined with SPE for purification purposes[17].

SPE is the most common applied purification step, either in the non-retentive mode or in retentive mode or a combination of the two modes. The choice for the SPE approach is seemingly not related to the type of matrix. In contrast, for extraction the alkaline digestion step is strongly related to the extraction of biological samples. Most studies which used an SPE purification step report reasonable recoveries (40% - 120%).

With regard to the used SPE sorbents, a few conclusions and assumptions are made. For instance, Weak Anion Exchange (WAX) SPE was considered to show higher selectivity and recovery for acidic analytes compared to e.g. Hydrophilic-Lipophilic Balance (HLB)

and florisil sorbents[7]. In a study by So et al.[34], the suitability of the HLB sorbent is questioned as recoveries below 30% were found for short chain PFCA (PFHxA, PFPeA, and PFBA). When WAX was used in combination with THF, low recoveries were found when an excess of THF was used[19]. Detailed methodology on the use of Strata X-AW (weak anion mixed mode), ENV (modified styrene-divinylbenzene polymer) and the non-retentive SPE columns containing Florisil was not found while the use of silica sorbent is assumed to reduce the recovery of PFOSA[31]. In an example of non-retentive SPE using graphitized carbon (Envi-Carb), it was stated that any π electrons in PFCA are strongly associated with the highly electronegative fluorine atoms and would not interact substantially with the graphitized carbon[35]. Even in the presence of a weak eluting solvent, the PFAS can be eluted while co-extracted constituents will be trapped on the column. Graphitized carbon has proven to be very effective and fit for purpose as dSPE[35,36,37]. Carbon in nanotube form (MWCNT) even has been used in combination with primary secondary amine (PSA) and/or C_{18} sorbent for a single step extraction and purification[5].

When using weak ion exchange SPE columns, the supernatant was acidified to protonate the secondary amines in these columns to promote the binding of PFAS. With a pKa value of 6[38], the WAX columns are almost fully protonated at pH 4 while the PFAS are in their anionic form[11,12,13,14,15]. During the wash step, with extraction solvents or water, the acidity is generally kept around pH 4 to keep the strong ionic bonding between anionic analytes and the cationic WAX sorbent. For elution, the polarity of the WAX column is deactivated by increasing the pH which neutralizes the secondary amines in the stationary phase. The ionic bond with the PFAS is consequently broken and the PFAS can be eluted with a suitable solvent, generally methanol.

TFC was used in two studies both in combination with an alkaline extraction. A benefit of this approach, is the possibility to implement this technique in an on-line clean-up setup[24,26]. Specific information on the performance of this setup was unfortunately not given.

The volumes of the obtained extracts are generally too high to reach the desired detection limits. In almost all studies, the volumes of the purified extracts were reduced until dryness, or a fixed volume using nitrogen or vacuum evaporators. However, the absorption of the analytes into the insoluble matrix components[18,39] and volatilization of analytes[40] have been assigned as critical parameters[41].

Methods: instrumental analysis

Instrumental analysis in the summarized studies are very consistent in terms of technique and columns and mobile phases. Liquid chromatography tandem mass spectrometry (LC-MS/MS) in the negative mode was nearly the sole technique used. The mass analyser varied from quadrupole to ion trap and time of flight. The LC-MS/MS approach probably prevailed as it allows for high sensitivity and selectivity.

Although C_{18} columns were generally used in varying lengths, diameter and particle size, four studies reported the use of a fluorinated column, (Flurosep RP Octyl[18], Hypersil GOLD PFP[22], Flurosep (unspecified)[23] and ACE C_{18} PFP[24,25]) for alternative selectivity.

Mobile phases were nearly all mixtures of water and methanol, and occasionally acetonitrile. Formic acid (FA), ammonium acetate (NH_4OAc), ammonium formate (AF) and 1-methyl piperidine were used to adjust and stabilize the pH. As PFAS tend to have very low pKa values, they remain in the negatively charged state during chromatography facilitating the ionization in mass spectrometry.

Automatization of purification

Laboratory work is more and more automated to improve precision, accuracy, sensitivity and robustness of methods while saving time for analysts, money on materials and chemicals and laboratory space[42]. The initial investment is often the obstacle that needs to be overcome, but in the longer term, this pays of financially and could improve the motivation of executing staff.

Many laboratories performing or willing to perform PFAS analysis already have experience with automatization of purification for dioxin analysis.

Figure 3 Promochrom SPE-03 8-channel high volume automated SPE system

The field of pesticides is also closely related and methods are similar if not the same. For the application of QuEChERS for pesticides analysis, automatization was of subject in recent times. Kaewsuya et al.[43] published in 2013 an automated dSPE approach while several manufacturers of robotics have developed systems for the automated analysis of pesticides.

Similar developments are occurring for PFAS analysis, and a validated automated system (fig. 3) for the analysis of PFAS in drinking water has been introduced[44]. The methods as well as the sample volume are variable and SPE methods can be automated using this system, making it also a viable system for the automatization of PFAS analysis in food.

Proposed method for extraction and automated purification

The proposed method (fig. 4) for food samples includes two extraction methods, 1) a straightforward SLE or
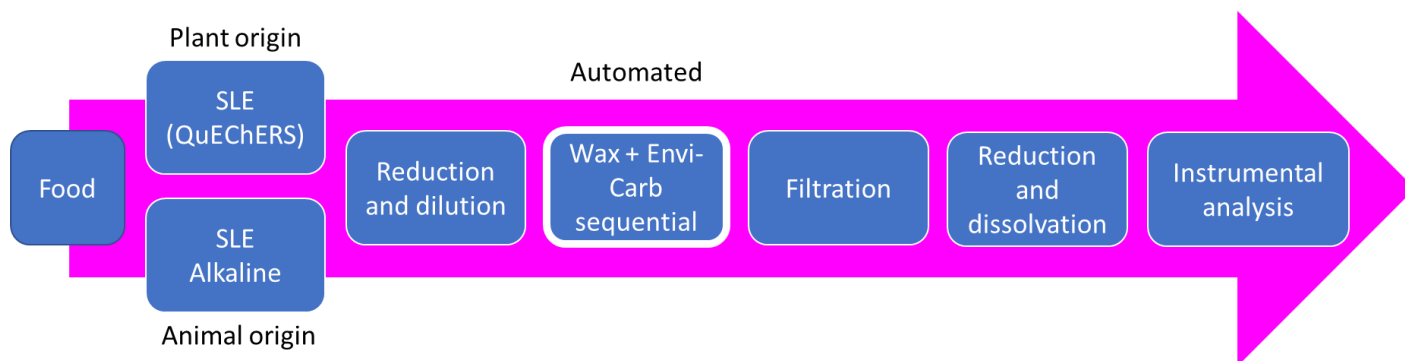
Figure 4 Proposed analytical procedure for the analysis of PFAS in food samples

QuEChERS method for samples of plant origin and 2) an alkaline SLE extraction for samples from animal origin which ensures the release of protein bound PFAS[9,10]. The extraction is followed by an in-line weak ion exchange SPE in retentive mode, in sequence with a graphitized carbon non-retentive SPE mode for increased selectivity[7]. The obtained extracts can be diluted with a vacuum evaporator (fig. 3). A more specific proposal for the sample preparation is described below.

Extraction

Weigh 2 g of sample in a 15 ml polypropylene (PP) tube and add internal standards (Cambridge Isotope Laboratories). Extract, by shaking thoroughly, twice with 5 ml acetonitrile:water (80:20 v/v) containing 10 mM KOH (alkaline) or 5 ml acetonitrile (SLE). Centrifuge and decant the supernatant carefully not to transfer any particulate matter. Combine both fractions in a 15 ml PP tube.

Reduction

Filter the obtained extracts with a Nylon filter and reduce the volume in, for instance, a Centrivap vacuum concentrator (Fig. 5) to 2 ml. Add until 10 ml with water (2% FA, pH 4).

Automated SPE

For automated SPE, a SPE-03 8-channel high volume automated SPE system from Promochrom is proposed. Equip the system with a weak anion exchange column coupled to a graphitized carbon column (commonly used WAX and Envi-Carb). Pre-condition the columns subsequently with 4 ml methanol (0.5% ammonia), 4 ml methanol and 4 ml water. Load the sample to the column and dry the cartridge. Rinse the sample container with 4 ml of 25 mM acetate buffer (pH 4) and load this to the column. Dry the column again. Wash

with 4 ml methanol and elute the PFAS with 8 ml methanol (0.5% ammonia).

Reduction

Reduce the volume in, for instance, a Centrivap vacuum concentrator (Fig. 5) to 0.5 ml. Dilute a part of the extract for analysis on the LC-MS/MS with an equivalent part of water containing 2 mM ammonium acetate.

Figure 5 Labconco Centrivap vacuum concentrator

Summary

Over the foundational and recent publications, methods were consistent and generally decent in terms of performance. In a previous review by Valsecchi et al.[10] many parts of the analysis were already well discussed. In this review some additional fundamental understandings were further described and were used to propose a suitable method for the analysis of PFAS using automated SPE.

As discussed before, automatization has proven to aid analysis in terms of precision, accuracy and sensitivity[42].As for dioxin analysis it also contributes to efficient use of laboratory space and analysts time.

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