AUTOMATED CLEAN-UP METHOD FOR THE PFAS DETERMINATION IN HEAVY POLLUTED WATERS

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Introduction

Perfluorinated organic compounds (PFCs) represents an emerging class of contaminants of great concern. They are globally distributed, environmentally persistent, bioaccumulative, and potentially harmful. The two most commonly used and PFCs found in the environment are Perfluoro-1-octanesulfonic acid (PFOS) and Perfluoro-n-octanoic acid (PFOA). These compounds have been commercially produced for decades and were employed in a wide range of industrial and consumer applications such as surface coatings on cooking pans, in making water and oil repellent products, in firefighting foams, and in food packaging[1,2].

The main exposure to these pollutants is through diet[3]. As a consequence of their persistence, bioaccumulation potential, toxicity, and widespread presence in environmental media, biota, and humans, the EU restricted their production[4] and PFOS was included in Annex B of the Stockholm Convention on Persistent Organic Pollutants[5].

The concerns about PFOS and PFOA increase the need to develop innovative and automated analytical methods for the determination of these pollutants. The most commonly applied method for PFCs purification is Solid Phase Extraction (SPE). This study points to the development of a simple method for the determination of PFOS, PFOA and PFDA (Perfluoro-n-decanoic acid) performed by an automated clean-up system.

Materials and methods

An autosampler loaded each sample to cartridges placed on one of three SPE purification modules (J2 Scientific, Missouri, USA).

Sample clean-up procedures were performed using two different cartridges to assess the recovery of PCFs on each:

- 500 mg of Styrene/Divinyl Benzene Copolymer Resin base material (Super-Clean ENVI-Chrom, Supelco, USA) according to the EPA method 537[6]
- 60 mg of polymeric reversed-phase adsorbent (Oasis HLB, Waters)

PFOS, PFDA and PFOA analytical determinations were performed by an isotopic dilution technique (LC/HRMS). Table 1 shows the list of native PFC standards (Wellington Laboratories, Canada), at a working concentration of 1 ng/ul, used in this study. The native PCFs were spiked at a rate of 10 ng/ml in 10mL of water. The water was further fortified with the 13C-labeled surrogate standards used for 'isotopic dilution' quantification, listed in Table 2 (Wellington Laboratories, Canada).

Super-Clean ENVI-Chrom cartridges were conditioned with 15 mL of methanol and 15 mL of Milli-Q water. The 10mL water samples were loaded on the cartridges at a flow rate of 5 mL/min and eluted with 20 mL of methanol at 5 mL/min.

Oasis HLB cartridges were conditioned with 10 mL of ethyl acetate, 10 mL of methanol and 10 mL of Milli-Q water. The 10mL water samples were loaded on the cartridges at a flow rate of 5 mL/min and eluted with 10 mL of ethyl acetate and 10 mL methanol containing 2 % ammonia

The eluate from each cartridge was manually concentrated to 100 μl and spiked with 13C-labelled standards (Wellington Laboratories, Canada), reported in table 3, and finally submitted to instrumental analysis.

Table 1: List of the Native PFCs standards

Compound	Abbreviation	CAS
Perfluoro-n-octanoic acid	PFOA	335-67-1
Perfluoro-n-decanoic acid	PFDA	335-76-2
Perfluoro-1-octanesulfonic acid	PFOS	207596-29-0

Table 2: List of the labelled PFCs in extraction standards

Compound	Abbreviation
Perfluoro-n-[$^{13}C_8$] octanoic acid	$^{13}C_8$-PFOA
Perfluoro-n-[1,2,3,4,5,6-$^{13}C_6$] decanoic acid	$^{13}C_6$-PFDA
Perfluoro-1-[$^{13}C_8$] octanesulfonic acid	$^{13}C_8$-PFOS

Table 3: List of the labelled PFCs in recovery standards

Compound	**Abbreviation**
Perfluoro-n-[1,2-$^{13}C_2$] octanoic acid	$^{13}C_2$-PFOA
Perfluoro-n-[1,2-$^{13}C_2$] decanoic acid	$^{13}C_2$-PFDA
Sodium perfluoro-1-[1,2,3,4-$^{13}C_4$] octanesulfonate	$^{13}C_4$-PFOS

Instrumental analysis:

Samples were analyzed using liquid chromatography-high resolution mass spectrometry (LC/HRMS). Detailed instrumental conditions are reported in table 4.

TABLE 4: Detailed instrumental conditions:

HPLC instrument:	Agilent 1200 series pumps and? autosampler
Column:	XBridge MS C18, 100x2.1 mm, 3.5 µm
Solvent A:	Ammonium acetate 10 mM in water
Solvent B:	Acetonitrile
Gradient:	From 1 to 99% B in 18 min; 99% B for 5 min
Flow:	200 µL/min
Injection volume	8 µL
HRMS instrument	Thermo Q Exactive
Ionization source	ESI, negative ions
Acquisition method:	Full scan MS (R=35000) and dd2-MS/MS (R=17500)

Isotopic dilution method was used for quantitative analyses using external calibration standard solutions for each substance. PFCs masses are listed in Table 5

Table 5: List of PFCs masses

Compound	**Mass 1**	**Mass 2**
PFOA	412.9664	368.9766
PFDA	512.9600	468.9702
PFOS	498.9302	
$^{13}C_8$-PFOA	420.9933	376.0001
$^{13}C_2$-PFOA	414.9731	369.9800
$^{13}C_6$-PFDA	518.9802	473.9870
$^{13}C_2$-PFDA	514.9667	469.9736
$^{13}C_8$-PFOS	506.9571	
$^{13}C_4$-PFOS	502.9436	

Results and discussion:

In order to evaluate the quantitative performance of the semi-automatic clean-up system, the 13C-labeled PFC's (which reflect the actual analyte recovery when using isotopic dilution techniques) mean recoveries and standard

Table 6: Mean recoveries and standard deviation of labeled PFCs

Sample	**$^{13}C_8$-PFOA**		**$^{13}C_6$-PFDA**		**$^{13}C_8$-PFOS**	
	Super-Clean ENVI-Chrom	**Oasis HLB**	**Super-Clean ENVI-Chrom**	**Oasis HLB**	**Super-Clean ENVI-Chrom**	**Oasis HLB**
1	82%	39%	51%	12%	66%	10%
2	89%	72%	81%	11%	88%	17%
3	86%	47%	89%	12%	82%	13%
4	92%	18%	99%	28%	98%	26%
5	97%	32%	69%	13%	72%	12%
6	100%	31%	65%	30%	80%	32%
7	92%	10%	68%	49%	71%	35%
Ave.	91%	36%	75%	22%	80%	21%
St.Dev.	6%	20%	16%	14%	11%	10%

deviations (%) obtained using the automated system and the two different SPE cartridges are shown in Table 6. Analyses were performed on 7 replicates.

Recoveries obtained from Super-Clean ENVI-Chrom are higher than those obtained from OASIS HLB, in particular using Super-Clean ENVI-Chrom $^{13}C_8$-PFOA recoveries range from 82 to 100%, $^{13}C_6$-PFDA range from 51 to 99%, $^{13}C_6$-PFOS range from 66 to 98%, while using OASIS HLB recoveries range from 10 to 72%, from 11 to 49% and from 10 to 35% respectively. Moreover the relative standard deviations of recoveries obtained from Super-Clean ENVI-Chrom are less than 30%, in agreement with the requirements of the reference method to evaluate the accuracy of the procedure[6]. In more detail recovery standard deviation is 6%, 16% and 11% for $^{13}C_8$-PFOA, $^{13}C_6$-PFDA and $^{13}C_6$-PFOS respectively.

In order to evaluate the precision of the method, the results of the replicate analysis from Super-Clean ENVI-Chrom, their average and standard deviation are reported in Table 7.

Table 7: results of replicate analysis from Super-Clean ENVI-Chrom

Sample	PFOA	PFDA	PFOS
1	11.1 ng/ml	9.1 ng/ml	9.4 ng/ml
2	12.0 ng/ml	8.1 ng/ml	9.7 ng/ml
3	11.1 ng/ml	8.9 ng/ml	8.8 ng/ml
4	12.1 ng/ml	9.9 ng/ml	10.0 ng/ml
5	11.0 ng/ml	9.8 ng/ml	8.1 ng/ml
6	11.6 ng/ml	8.5 ng/ml	9.6 ng/ml
7	11.3 ng/ml	8.8 ng/ml	9.4 ng/ml
Ave.	11.5 ng/ml	9.0 ng/ml	9.3 ng/ml
St. Dev	0.4 ng/ml	0.7 ng/ml	0.6 ng/ml
St.Dev/True Value	4%	7%	6%

The analytical results obtained are in agreement with the precision requirements of the reference method, showing a standard deviation less than 20% of the true value. In particular the standard deviation obtained in 4%,7% and 6% of the true value for PFOA, PFDA and PFOS

Examples of chromatograms of PFOA and PFOS obtained using Super-Clean ENVI-Chrom HLB are reported in Fig.1, Fig.2 respectively.

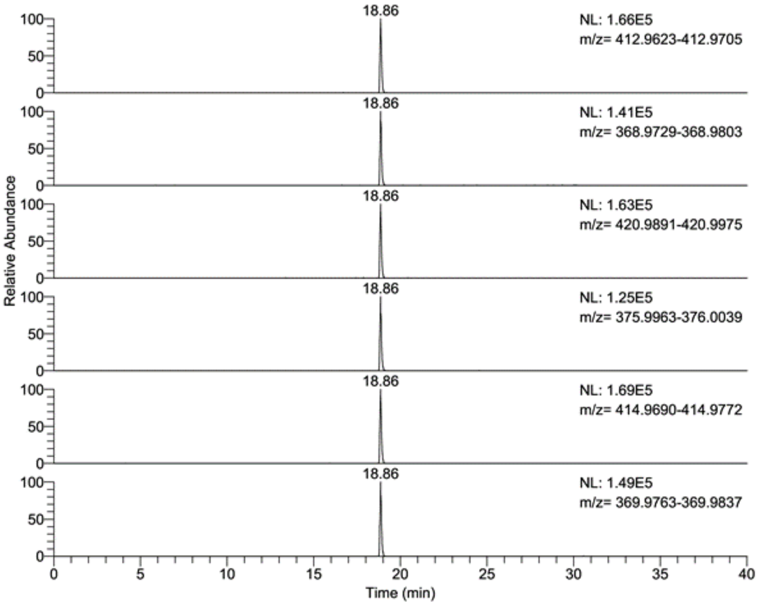

Figure 1: Example of chromatogram of PFOA obtained from Super-Clean ENVI-Chrom.

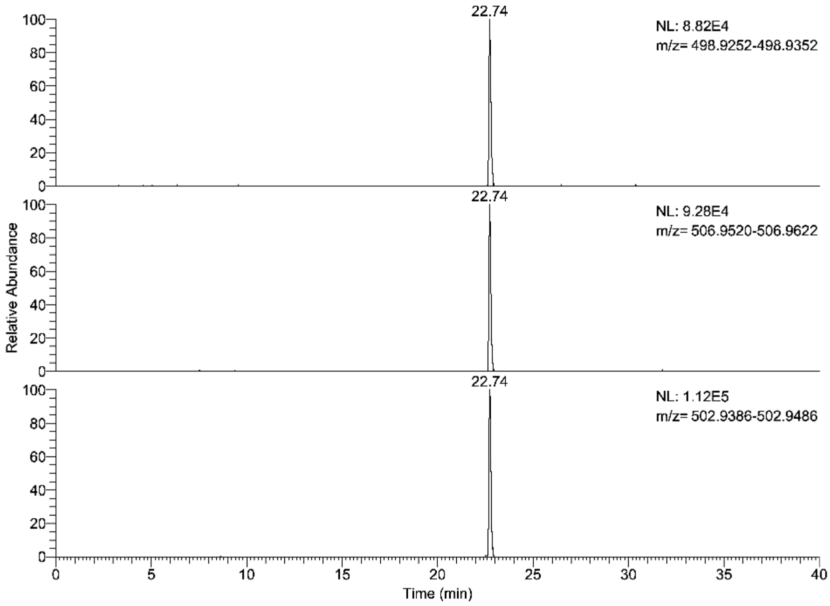

Figure 2: Example of chromatogram of PFOS obtained from Super-Clean ENVI-Chrom.

Our next goal is complete automation of the cleanup procedure. The current automated system is able to send each fraction eluted from the SPE to an in-line evaporation system where they are concentrated then directly injected into vials, prior to the instrumental analysis. Particular attention will be paid to the PFCs behavior during the evaporation step.

Acknowledgemets:
We want to thanks J. Salmons and M. Tanner, from J2 Scientific Llc, for their revision of the paper.

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