

Development of Automated Screening and Quantitation Approach on Novel On-Line SFE-SFC-MS/MS Platform – (I) For 23 Restricted Perfluorocompounds in Textiles

ASMS 2016 MP 283

Jie Xing, Jun Xiang Lee, Peiting Zeng and Zhaoqi Zhan
Application Development & Support Centre,
Shimadzu (Asia Pacific) Pte Ltd, 79 Science Park Drive,
Singapore

Development of Automated Screening and Quantitation Approach on Novel On-Line SFE-SFC-MS/MS Platform – (I) For 23 Restricted Perfluorocompounds in Textiles

Introduction

Supercritical Fluid Chromatography (SFC) with carbon dioxide as eluent has attracted attention recently because of its advantages in low running cost, non-toxicity and wider coverage of analytes in terms of polarity. The combination of SFC with SFE (E=Extraction) has extended the applications to fully-automated sample pre-treatment and analysis as demonstrated by the Nexera UC system introduced by Shimadzu recently. The novel SFE-SFC-MS/MS system has been used successfully for

analysis of 510 residual pesticides in agricultural products [1]. One of the main advantages of the Nexera UC platform allows to set up on-line method to analyse directly different types and forms of un-pretreated samples. We describe the development of an approach on the Nexera UC platform, aiming at screening and quantitation of 23 perfluorocompounds (PFCs) listed under the Restricted Substance List (RSL) in textile, leather and consumer goods industries [2].

Experimental

A total of 23 PFCs and 2 internal standards (IS), M-PFOS and M-PFOA (refer to Table 2) were obtained from Sigma Aldrich, Wellington Laboratories and Apollo Scientific [3]. Textile samples were cut into smaller pieces and weighed. The sample (60 mg) was loaded into a 0.2 mL stainless steel SFE vessel tightly before proceeding to online-SFE-SFC-MSMS analysis. A schematic diagram of

the Nexera UC system employed in this study is shown in Figure 1. The system can be operated for on-line SFE-SFC-MS/MS experiments or only for SFC-MS/MS analysis. The mobile phase is supercritical fluid CO₂, with addition of organic modifier like MeOH. Thus, both isocratic and gradient elution modes can be chosen. The details of the analytical conditions are compiled into Table 1.

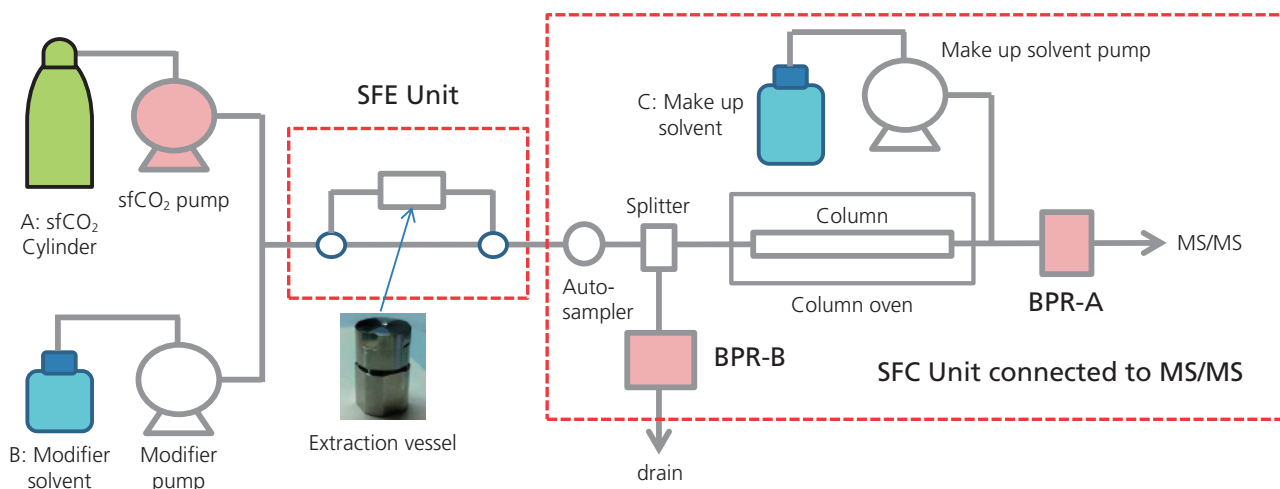


Figure 1: Schematic diagram of Nexera UC system for SFE-SFC-MS/MS analysis of un-pretreated samples

Development of Automated Screening and Quantitation Approach on Novel On-Line SFE-SFC-MS/MS Platform – (I) For 23 Restricted Perfluorocompounds in Textiles

Table 1: Analytical conditions of 23 PFCs and 2 internal standard on Nexera UC with LCMS-8050

Column	: Shim-pack UC-X Sil (250 mmL. x 2.1mm I.D., 3µm)
Flow Rate	: 2.0 mL/min 0.2 mL/min (make up pump of MS)
Mobile Phase	: A : Supercritical Fluid Carbon dioxide (sfCO ₂) B : Methanol with 5 mM ammonium formate C : Methanol with 5 mM ammonium formate
Temp.	: Column Oven: 40°C; SFE unit: 40°C or RT
Injection vol.	: SFC: 5 µL; SFE-SFC: 200 µL or 3% split ratio
Elution Mode	: Gradient elution, LC program 7 minute 0%B (0.00mins to 0.30mins) → 50%B (4.00mins to 4.50mins) → 0%B (4.70mins to 6.00mins)
Interface	: ESI
MS mode	: Negative
Block Temp.	: 400°C
DL Temp.	: 250°C
Interface Temp.	: 300°C
CID Gas	: Ar (270kPa)
Nebulizing Gas Flow	: N ₂ , 3 L/min
Drying Gas Flow	: N ₂ , 10 L/min
Heating Gas Flow	: 0 Air, 10 L/min

Results and Discussion

Establishment of SFC-MS/MS method

A SFC-MS/MS method was established for the targeted 23 PFCs with 2 IS first. A Shim-pack UC-X Sil column was used and a gradient elution program was adopted. Two MRM transitions (if available) were used for each PFC, one as quantifier ion and the other for confirmation. The SFC-MS/MS chromatograms and calibration curves (only PFOA and PFOS are displayed) are shown in Figure 2. Calibration curves were built based on the two internal

standards with linearity ($r^2 > 0.97$) for all 23 PFCs (Table 2). Instead of using the concentration, absolute amount (pg) was used for the calibration curves. The LOQ ranges from 0.03 ~ 1 ng/mL while the LOD ranges from 0.01 ~ 0.32 ng/mL. The repeatability of the method was evaluated at two concentrations, 5 and 25 pg. The %RSD results of the post-spiked samples are tabulated in Table 2.

Development of Automated Screening and Quantitation Approach on Novel On-Line SFE-SFC-MS/MS Platform – (I) For 23 Restricted Perfluorocompounds in Textiles

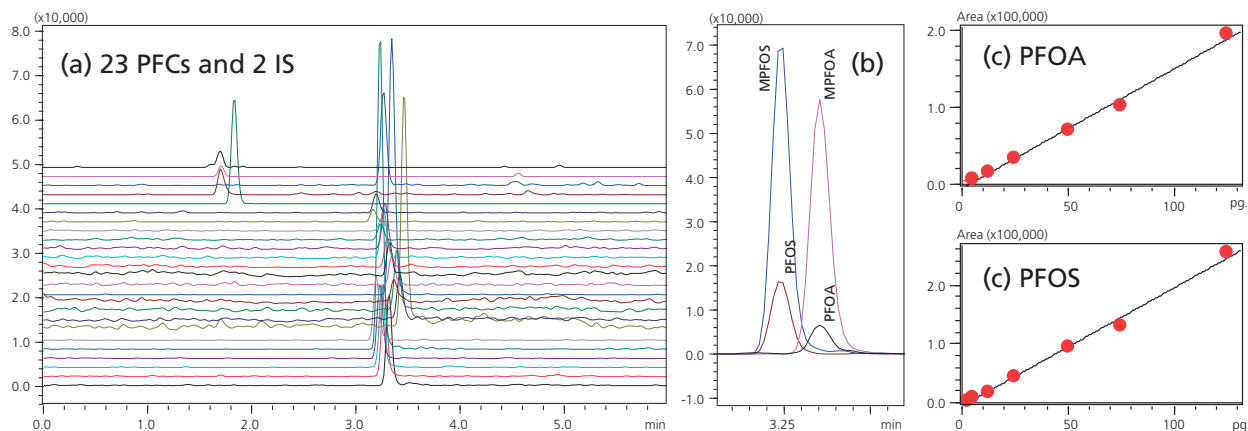


Figure 2: (a) MRM chromatograms of 23 PFC mixed standards with 2 IS, 5 pg for each compound;
(b) Zoomed MRM peaks of PFOA and PFOS with their IS;
(c) Calibration curves of PFOA (5~125 pg) and PFOS (2.5~125 pg) based on quantifier ions as shown in table 3.

Table 2: Calibration curves and performance values of the MRM method for quantitative determination of 23 PFCs on SFC-MS/MS.
Absolute amounts (in pg) of analytes are used for convenience (injection volume: 5 μ L)

No.	Name	RT (min)	MRM transition	Range (pg)	R ²	LOD (pg)	LOQ (pg)	RSD (%), n=6	
								(5 pg)	(25 pg)
1	N-EtFOSA-M	1.613	526.10>169.00	5 ~ 125	0.9732	< 2.5	5	34.4	32.3
2	N-MeFOSA-M	1.692	512.00>169.15	2.5 ~ 125	0.9966	< 2.5	5	47.1	18.1
3	H4PFUnA	1.695	491.10>367.00	2.5 ~ 125	0.9877	1.2	3.6	59.9	20.7
4	FOSA	1.831	498.00>77.90	2.5 ~ 125	0.9926	< 2.5	5	23.5	7.9
5	PFODA	3.171	913.00>868.90	2.5 ~ 125	0.9916	0.45	1.3	43.9	12.1
6	PFDHxA	3.2	813.00>768.80	2.5 ~ 125	0.9985	0.25	0.8	16.3	8.3
7	PFDS	3.201	599.00>80.00	2.5 ~ 125	0.9942	0.1	0.3	21.5	8.4
8	PFTeDA	3.23	712.90>668.90	5 ~ 125	0.9947	1.55	4.7	14.4	10.4
9	PFOS	3.233	499.00>79.90	2.5 ~ 125	0.9881	0.1	0.35	21	9.5
10	PFHpS	3.253	449.00>79.85	2.5 ~ 125	0.9924	0.15	0.45	15.6	9.6
11	PFTTrDA	3.257	663.00>619.00	2.5 ~ 125	0.9986	0.75	2.2	23.7	20.7
12	PFDoA	3.265	612.90>569.00	2.5 ~ 125	0.9943	0.5	1.6	13.5	13
13	PF-3,7-DMOA	3.266	469.05>269.00	2.5 ~ 125	0.9918	0.15	0.4	22.3	9.1
14	PFHxS	3.278	399.00>79.90	2.5 ~ 125	0.9923	0.1	0.35	26.9	5.5
15	PFUdA	3.289	563.00>519.00	2.5 ~ 125	0.9975	0.5	1.55	17.7	8.1
16	PFDA	3.307	512.80>468.90	2.5 ~ 125	0.9946	0.75	2.3	25.4	8.3
17	PFBS	3.322	298.80>79.90	2.5 ~ 125	0.9951	0.15	0.45	18.9	8.8
18	PFNA	3.329	462.90>418.90	5 ~ 125	0.9908	1.5	4.55	23.4	12.3
19	PFOA	3.354	413.10>369.10	5 ~ 125	0.9832	1.6	4.8	15	6.1
20	PFHpA	3.38	313.10>269.05	5 ~ 125	0.996	1.25	3.8	14.4	11.8
21	PFHxA	3.399	263.00>219.00	2.5 ~ 125	0.9951	0.3	0.95	18	13.8
22	PFPeA	3.43	212.90>168.95	2.5 ~ 125	0.996	0.35	1.1	18.7	11.4
23	PFBA	3.466	363.10>319.00	2.5 ~ 125	0.9949	0.15	0.5	11.7	8.7
IS1	M-PFOS	3.235	503.00>79.85	10				Not Available	
IS2	M-PFOA	3.349	416.90>372.00	10				Not Available	

Development of Automated Screening and Quantitation Approach on Novel On-Line SFE-SFC-MS/MS Platform – (I) For 23 Restricted Perfluorocompounds in Textiles

Development of on-line SFE-SFC-MS/MS approach

Next, an on-line SFE-SFC-MS/MS approach was developed based on the SFC-MS/MS method established. The mixed standard samples for calibration curve construction could be introduced into the system only by pre-loading them onto filter papers. 50 μL of mixed standard solution was dropped onto half filter paper (recommended by Shimadzu for Nexara UC use) and left it to dryness under N_2 flow before loading into the SFE vessel (0.2 mL). The results are shown in Figure 3 and Table 3 (columns 1-7). First, with on-line SFE, the elution peaks of the PFCs become broader and RTs delay slightly (about 0.2 min) in comparison with SFC-MS/MS chromatograms. This peak broadening and delay are due to the larger delay volume by the SFE vessel, needles and the tubing from SFE to column, which caused differences in peak areas and intensity. For direct quantitation of PFCs using on-line SFE-SFC-MS/MS,

calibration curves must be established on SFE-SFC-MS/MS too (Figure 3(c) & Table 3 (left).

If we compare the peak areas obtained on SFE-SFC-MS/MS and SFC-MS/MS, system recovery of the SFE-SFC-MS/MS could be estimated (see Table 3, columns 8-11). Although this system recovery may not be highly accurate, it can be used as a reference to understand the performance of the on-line SFE-SFC-MS/MS for quantitation. The average system recovery measured at the absolute loading amounts of 25 μg and 50 μg are 90 % and 74 %, respectively. The average repeatability (RSD%, $n=4$) of the system with loading amounts of 25 μg and 50 μg are 12 % and 14 %, respectively. It is worth noting that all of the analysis runs shown above are under the condition without splitting of the flow (sfCO_2 and MeOH) from SFE to SFC-MS/MS (PBR-B was set to zero flow to drain).

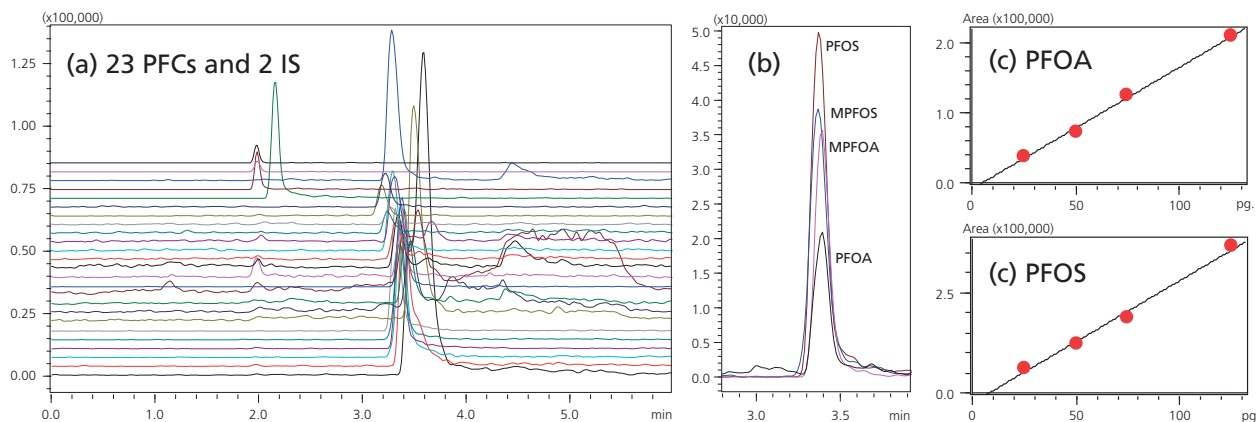


Figure 3: (a) MRM chromatograms of 23 PFC mixed standards with 2 IS on filter paper, 25 μg for each compound.
(b) Separate display of MRM peaks of PFOA, M-PFOA (IS, 10 μg), PFOS and M-PFOS (IS, 10 μg),
(c) Calibration curves of PFOA and PFOS on SFE-SFC-MS/MS.

Development of Automated Screening and Quantitation Approach on Novel On-Line SFE-SFC-MS/MS Platform – (I) For 23 Restricted Perfluorocompounds in Textiles

Table 3: Calibration curves of 23 PFCs (on filter paper in 0.2mL SFE vessel) of on-line SFE-SFC-MS/MS method (left table); System recovery estimated by comparison with SFC-MS/MS method (right Table)

ID#	Name	m/z	Ret. Time	Range (pg)	R ²	Accuracy (%)	25 pg (spiked)		50 pg (spiked)	
							Recovery %	RSD% (n=4)	Recovery %	RSD% (n=4)
1	N-EtFOSA	526.10>169.00	1.636	25 - 125	0.964	103	137.3	19.4	102.0	12.4
2	N-MeFOSA	512.00>169.15	1.692	25 - 125	0.9668	103.6	89.2	40.1	49.4	33.0
3	H4PFUnA	491.10>367.00	1.694	25 - 125	0.9956	101.2	87.8	24.2	51.6	26.0
4	FOSA	498.00>77.90	1.828	25 - 125	0.9978	100.7	61.0	18.6	40.2	23.8
5	PFODA	913.00>268.95	3.15	25 - 125	0.9946	101.5	91.2	16.9	80.7	20.3
6	PFDHxA	813.00>169.10	3.163	25 - 125	0.988	102.3	83.9	10.7	74.1	14.5
7	PFDS	599.00>98.80	3.3	25 - 125	0.9998	99.7	80.0	11.3	68.9	5.7
8	PFTeDA	712.90>219.20	3.196	25 - 125	0.9968	101.1	104.1	7.7	98.0	13.2
9	PFOS	499.00>98.50	3.344	25 - 125	0.993	101.5	92.6	5.4	75.7	3.5
10	PFHpS	449.00>99.05	3.344	25 - 125	0.9942	101.4	91.9	1.1	77.5	3.6
11	PFTrDA	663.00>169.10	3.198	25 - 125	0.9956	101.3	72.8	10.0	71.5	19.2
12	PFDoA	612.90>319.00	3.22	25 - 125	0.9947	99.9	101.3	12.0	87.1	10.2
13	PF-3,7-DMOA	469.05>68.80	3.229	25 - 125	0.9949	101.5	98.0	8.4	74.4	5.2
14	PFHxS	399.00>79.90	3.401	25 - 125	0.9958	100.7	87.5	1.1	78.0	4.4
15	PFUdA	563.00>268.90	3.265	25 - 125	0.995	98.7	84.4	1.1	76.6	17.7
16	PFDA	512.80>169.00	3.262	25 - 125	0.9905	101.3	106.1	4.9	89.6	14.6
17	PFBS	298.80>98.80	3.357	25 - 125	0.9912	101.6	89.1	7.9	69.0	8.1
18	PFNA	462.90>168.90	3.31	25 - 125	0.9986	100.6	103.6	8.2	78.8	10.4
19	PFOA	413.10>168.85	3.36	25 - 125	0.9972	100.8	97.8	7.7	87.3	15.0
20	PFHpA	363.10>169.05	3.375	25 - 125	0.9904	101.7	95.9	11.0	83.6	4.0
21	PFHxA	313.10>119.00	3.397	25 - 125	0.9776	100.3	76.6	8.5	74.1	11.4
22	PFPeA	263.00>219.00	3.431	25 - 125	0.9991	100.6	87.0	7.1	62.4	12.4
23	PFBA	212.90>168.95	3.446	25 - 125	0.9997	100	53.0	25.7	41.3	32.5

Automated SFE-SFC-MS/MS approach for analysis of PFCs in textiles samples

There are two extraction modes in the on-line SFE stage, static extraction and dynamic extraction with sfCO₂ or a mixture of sfCO₂ and MeOH (modifier). For more effective extraction, static extraction is performed first for 4 mins at 40 °C, followed by dynamic extraction for 2 mins. Figure 4 shows an example of the analysis of un-pretreated clothing sample and the same sample spiked with mixed PFCs (25 pg each on 60 mg of sample). The results shows that there are no PFC detected in the textile sample, which is in agreement with the offline LC/MS/MS analysis results [4]. On the other hand, all of the 23 PFCs spiked into the

sample were detected. The amount of PFCs spiked are equal to 0.42 ng/g (ppb), where detection sensitivity meets the requirement for monitoring PFOA and PFOS in consumer products [2]. This preliminary finding reveals the potential possibility of using on-line SFE-SFC-MS/MS as a new automated screening and quantitation system in analysis of un-pretreated samples directly. However, further studies of this novel approach for PFCs and other targeted analytes in various consumer samples are under investigation for optimizing the SFE conditions and improving the recovery for samples with complex matrix.

Development of Automated Screening and Quantitation Approach on Novel On-Line SFE-SFC-MS/MS Platform – (I) For 23 Restricted Perfluorocompounds in Textiles

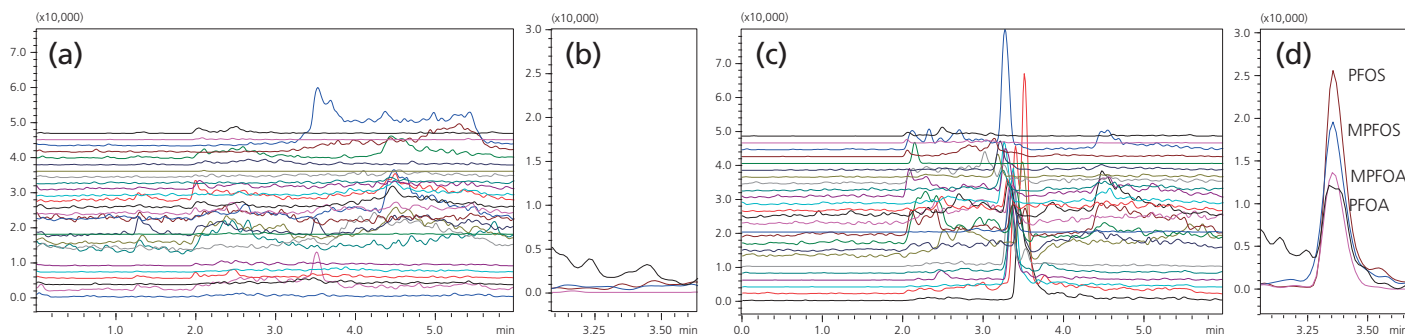


Figure 4: MRM chromatograms for screening of 23 PFCs in a textile sample (a-b) and in the same sample spiked with mixed 23 PFCs standards with 2 IS, 25 pg for each compound (c-d). Only dynamic extraction was used.

Conclusions

In this study, a new analytical approach on the novel SFE-SFC-MS/MS platform was developed for analysis of 23 PFCs including PFOA and PFOS spiked in textiles. The results indicate that the new approach is potentially possible for screening and quantitation of targeted

analytes in un-pretreated solid samples directly. The detection sensitivity of the 23 PFCs spiked in clothing sample achieved is at the level of 25 pg or 0.42 $\mu\text{g}/\text{kg}$. However, validation of the SFE-SFC-MS/MS approach for actual samples are not carried out yet.

References

1. Shimadzu Application News No LAAN-A-LC-E273, **Using the Nexera UC Online SFE-SFC-MS System to Analyze Residual Pesticides in Agricultural Products (2015)**.
2. I. van der Veen, J. M. Weiss, A. C. Hanning, J. de Boer and P. E. Leonards, *Talanta* 147 (2016), 8-15.
3. Wellington Laboratories, "Quick Reference Guide for Perfluoroalkyl Compounds", <http://www.well-labs.com>
4. J. X. Lee, Z. Sun, J. Xing, J. Y. Tan and Z. Zhan, ASMS 2016, Poster Session TP 485.

Disclaimer: The products and applications in this poster presentation are intended for Research Use only (RUO). Not for use in diagnostic procedures.

First Edition: June, 2016