Semi-automated analysis of PFAS from human serum

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Application benefits

- Successful automated determination of 36 per- and polyfluoroalkyl substances from human serum
- High recovery rates were achieved with a bilayer dual-phase SPE in CHROMABOND® PFAS Multi 96-Monoblock DE format
- Fast, sensitive and high reproducible analysis on Resolvex® A200 positive pressure processor (Tecan, Männedorf, Switzerland)
- Fast and sensitive HPLC analysis on a NUCLEODUR® Phenyl-Hexyl column

MN products

REF 738510.010M
CHROMABOND® PFAS Multi 96-Monoblock DE, 10 mg

REF 760576.20
EC 100/2 NUCLEODUR® Phenyl-Hexyl, 3 µm

REF 760573.20
EC 50/2 NUCLEODUR® Phenyl-Hexyl, 3 µm

REF 702402
Screw closure, N 9, PP, blue, center hole, silicone white/polyimide orange, 1 mm, fluorine-free

REF 702009
Screw neck vial, N 9, 11.6 × 32.0 mm, 0.3 mL, inner cone, PP transparent

Keywords

CHROMABOND® PFAS Multi 96-Monoblock DE, Resolvex® A200, positive pressure, PFAS, semi-automating SPE, human serum, LC-MS/MS

Introduction

The analysis of PFAS in environmental and food samples has developed enormously in recent years. The focus of public health is therefore on examining bioaccumulation in the human body. PFAS are widespread in the environment and in everyday consumer products, including our drinking water supplies as shown in figure 1 [1]. PFAS are associated with many negative aspects to human health [2, 3, 4].

Health Concerns of PFAS:

- Affects growth, learning, behavior
- Endocrine interference
- Increase cholesterol levels
- Affect the immune system
- Increase the risk of cancer
- Infertility

Therefore, understanding the effects of PFAS on the human body requires quantitative tools that can accurately and precisely detect low levels of PFAS in biological fluids.

The need for automated high-throughput methods in sample preparation is growing as part of PFAS bioaccumulation studies and monitoring projects.

In this application note, the suitability of the Resolvex® A200 positive pressure processor (Tecan, Männedorf, Switzerland) was investigated for semi-automating SPE of PFAS using the CHROMABOND® PFAS Multi 96-Monoblock DE. The workflow uses automated pressure profiles for the conditioning, washing and elution steps to yield high recovery rate for PFAS analytes. This semi-automated workflow allows users to achieve high sample throughput with very good reproducibility. Finally, the extracts are analyzed using HPLC-MS/MS on a NUCLEODUR® Phenyl-Hexyl column.

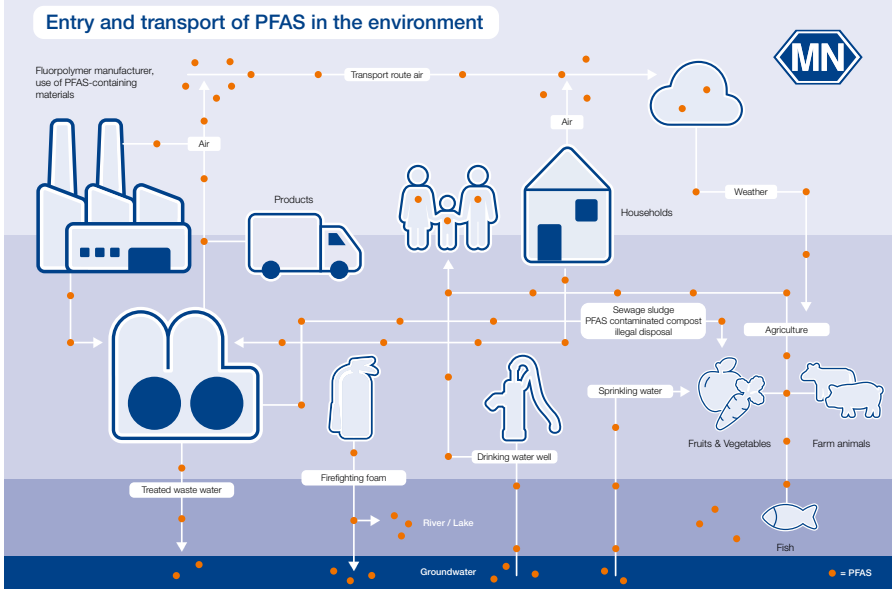


Figure 1: Entry and transport of PFAS in the environment.

Materials and Methods

The Resolvex® A200 (Figure 2A) is a compact standalone system for automated solid phase extraction. Combining programmable eight-channel dispensing with positive pressure processing, it offers optimized sample preparation with minimal manual intervention (Figure 2B). The presented solid phase extraction procedure takes about 20 minutes for a sample number of 96. The used deep edge (DE) plate design fits by height and width. A contamination through splashing is therefore not possible.

Figure 2A
Resolvex® A200 (Tecan, Männedorf, Switzerland)

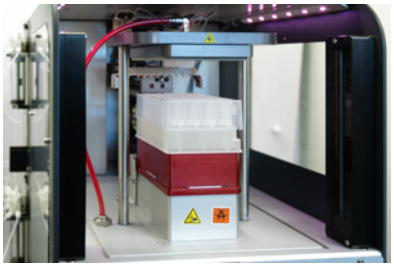


Figure 2B
Setup of semi-automated SPE

Figure 2C
CHROMABOND® PFAS Multi 96-Monoblock DE, 10 mg

Sample preparation

As part of sample preparation, protein precipitation must first take place. The protein precipitation ratio must be optimized so that possible PFAS protein binding in the serum samples are destroyed. After a first comparison of acetonitrile and methanol as protein precipitation agent, each in a volume ratio of 1:3 serum to solvent, acetonitrile has proven to be more effective. The organic solvent acetonitrile showed better results, particularly for long-chain PFAS.

Human serum sample was spiked with mass-labelled standard solution (and native standard solution for determination of recovery rate). An aliquot of 50 µL sample was added to 150 µL acetonitrile, vortexed and centrifuged at 13500 rpm for 10 minutes (VWR Microstar 12). The protein-precipitated sample was then diluted with 800 µL of 1 % formic acid. The mixture was ready for the SPE.

Solid phase extraction - SPE Procedure

The semi-automated SPE includes 5 steps as shown in the workflow (Figure 3). The solvents for conditioning the plate were provided in reservoir bottles and applied using the A200's dispensing unit. After each addition step, the solvent was forced through the sorbent bed using an optimized positive pressure profile. The conditioning of the CHROMABOND® PFAS Multi 96-Monoblock DE started with 500 µL of 2 % ammonia in methanol, continued then with 500 µL of methanol and ended with 500 µL of 1 % formic acid in water. The sample was applied in 2 portions of 500 µL each. To prevent adsorption effects of PFAS on the vessel walls, the sample vessel was rinsed with 200 µL 1 % formic acid in water and rinsing solvent was applied to the plate. Then, the plate was rinsed with a volume of 500 µL of acetonitrile:water (1:3, v/v). After replacing the spacer with a collection tray, the elution was done with 200 µL 2 % ammonia in methanol in 2 portions of 100 µL. Before injection into the LC/MS/MS system, the eluate was neutralized with acetic acid.

Figure 3: Workflow of human serum sample analysis

Line Item	Step	Time	Description	Processed
1	Preparing the instrument	1 min	Assemble stack and transfer into Resolvex® A200	Manual
2	Conditioning	2 min	500 µL of 2 % ammonia/methanol, apply low pressure up to 10 % for 45 sec	Resolvex® A200
3	Conditioning	2 min	500 µL of methanol, apply low pressure up to 10 % for 45 sec	Resolvex®A200
4	Conditioning	2 min	500 µL of 1 % formic acid/water, apply low pressure up to 15 % for 45 sec	Resolvex® A200
5	Load sample	1 min	Dispense 500 µL into the SPE cartridge	Manual
6		1 min	Apply high pressure up to 15 % for 45 sec	Resolvex® A200
7	Load sample	1 min	Dispense 500 µL into the SPE cartridge	Manual
8		1 min	Apply high pressure up to 15 % for 45 sec	Resolvex® A200
9	Rinse reservoir	2 min	Rinse with 200 µL of 1 % formic acid/water	Manual
10		1 min	Apply high pressure up to 20 % for 15 sec	Resolvex® A200
11	Wash	1 min	500 µL of acetonitrile/water (1:3), apply high pressure up to 20 % for 15 sec	Resolvex® A200
12	Preparing for collection	1 min	Replacing the waste barrier with a collcetion tray	Manual
13	Elute	2 min	100 µL of 2 % ammonia/methanol, apply low pressure up to 65 % for 20 sec	Resolvex® A200
14	Elute	2 min	100 µL of 2 % ammonia/methanol, apply low pressure up to 65 % for 20 sec	Resolvex® A200
15	Forward for analysis	1 min	Disassemble stack from Resolvex® A200, forward collection to LCMS	Manual

Table 1: Resolvex® A200 (Tecan, Männedorf, Switzerland) workflow for SPE of PFAS from human serum

Analysis by HPLC-MS/MS

Chromatographic conditions

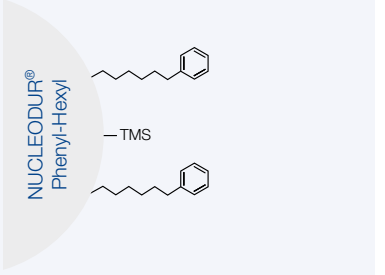
DELAY Column	EC 50/2 NUCLEODUR® Phenyl-Hexyl (REF 760573.20)
Column	EC 100/2 NUCLEODUR® Phenyl-Hexyl, 3 µm (REF 760576.20)
Eluent A	5 mM ammonium acetate in water
Eluent B	5 mM ammonium acetate in methanol
Gradient	hold 40 % B for 1 min, in 8 min from 40 % B to 95 % B, hold 95 % B for 3 min, in 0.1 min to 40 % B, hold 40 % B for 2.9 min
Flow rate	0.3 mL/min
Temperature	40 °C
Injection volume	2 µL

MS Conditions

Acquisition mode	SRM
Interface	ESI
Polarity	negative
Curtain Gas	30
Collision Gas	medium
Ionspray Voltage	-4500 V
Temperature	400 °C
Ion Source Gas 1	50
Ion Source Gas 2	60
Detection Window	60 sec

Good to know

Structure of NUCLEODUR® Phenyl-Hexyl



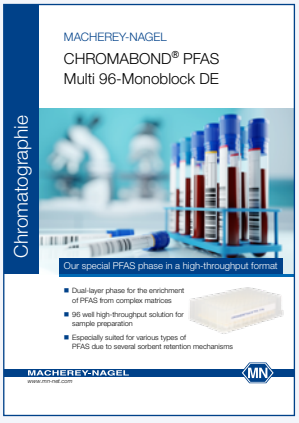
MRM transitions

Analyte	Abbreviation	CAS number	Q1 mass [Da]	Q3 mass [Da]	Retention time [min]
Perfluoro-*n*-butanoic acid	PFBA	375-22-4	212.90	168.80	1.76
Perfluoro-3-methoxypropanoic acid	PFMPA	377-73-1	229.00	85.00	2.15
3-Perfluoropropyl propanoic acid	3:3FTCA	356-02-5	241.00	177.00	3.00
Perfluoro-*n*-pentanoic	PFPeA	2706-90-3	262.88	219.00	3.15
Perfluoro-*n*-butanesulfonic acid	PFBS	375-73-5	298.93	98.90	3.50
Perfluoro-4-methoxybutanoic acid	PFMBA	863090-89-5	279.00	85.00	3.60
Perfluoro(2-ethoxyethane)sulfonic acid	PFEESA	113507-82-7	315.00	135.00	4.12
Nonafluoro-3,6-dioxaheptanoic acid	NFDHA	151772-58-6	295.00	201.00	4.35
Perfluoropentansulfonic acid	PFPeS	2706-91-4	348.85	80.00	4.75
Perfluoro-*n*-hexanoic acid	PFHxA	307-24-4	312.91	268.80	4.76
Hexafluoropropylene oxide dimer acid	HFPO-DA	13252-13-6	284.99	168.70	5.00
Perfluoro-*n*-hexansulfonic acid	PFHxS	355-46-4	398.94	79.80	5.54
Perfluoro-*n*-heptanoic acid	PFHpA	375-85-9	362.93	318.80	5.57
2H,2H,3H,3H-Perfluorooctanoic acid	5:3FTCA	914637-49-3	341.00	237.00	5.60
4,8-Dioxa-3H-perfluorononanoic acid	ADONA	919005-14-4	376.90	250.70	5.74
Perfluoro-*n*-octanoic acid	PFOA	335-67-1	412.91	369.00	6.35
Perfluoro-*n*-heptanesulfonic acid	PFHpS	375-92-8	448.93	79.80	6.41
Perfluoro-*n*-nonanoic acid	PFNA	375-95-1	462.89	418.90	7.00
Perfluoron-octanesulfonic acid	PFOS	1763-23-1	498.84	79.90	7.00
3-Perfluoroeptyl propanoic acid	7:3FTCA	812-70-4	441.00	317.00	7.20
9-Chlorohexadecafluoro-3-oxanonane -1-sulfonic acid	9Cl-PF3ONS	736606-19-6	630.75	350.70	7.43
Perfluoro-*n*-decanoic acid	PFDA	33-5-76 – 2	512.84	468.90	7.58
Perfluorononanesulfonic acid	PFNS	68259-12-1	548.81	79.90	7.59
N-methyl perfluorooctanesulfonamidoacetic acid	*N*-MeFOSAA	2355-31-9	569.80	418.90	7.90
Perfluoro-*n*-undecanoic acid	PFUnDA	2058-94-8	562.80	518.90	8.02
Perfluoro-*n*-decanesulfonic	PFDS	335-77-3	598.79	79.90	8.02
N-ethyl perfluorooctanesulfonamidoacetic acid	*N*-EtFOSAA	2991-50-6	583.81	418.80	8.14
11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	11CL-PF3OUdS	763051-92-9	630.74	451.00	8.34
Perfluoro-*n*-dodecanoic acid	PFDoDA	307-55-1	612.79	568.90	8.40
Perfluorododecanesulfonic acid	PFDoS	79780-39-5	698.72	80.00	8.72
Perfluoro-*n*-tridecanoic acid	PFTrDA	72629-94-8	662.77	618.90	8.73
N-methyl perfluorooctanesulfonamide	*N*-MeFOSA	31506-32-8	512.00	169.00	8.84
N-ethyl perfluorooctanesulfonamidoethanol	NEtFOSE	1961-99-2	630.00	59.00	8.87
Perfluoro-*n*-tetradecanoic acid	PFTeDA	376-06-7	712.77	668.80	9.03
N-ethyl perfluorooctanesulfonamide	*N*-EtFOSA	4151-50-2	526.00	169.00	9.09
N-methyl perfluorooctanesulfonamidoethanol	NMeFOSE	24448-09-7	616.00	59.00	9.11
Surrogates					
Perfluoro-($^{13}C_4$)butanoic acid	M4PFBA		216.94	171.90	1.75
Perfluoro-($^{13}C_5$)pentanoic acid	M5PFPeA		267.97	222.90	3.13
Sodium perfluoro-(2,3,4 – $^{13}C_3$)butanesulfonate	M3PFBS		301.89	98.90	3.48
Perfluoro-(1,2,3,4,6 – $^{13}C_5$)hexanoic acid	M5PFHxA		318.00	272.80	4.53
Tetrafluoro-2-heptafluoropropoxy-$^{13}C_3$-propanoic acid	M3HPFO-DA		287.00	169.00	4.98
Perfluoro-(1,2,3,4 – $^{13}C_4$)heptanoic acid	M4PFHpA		366.95	321.80	5.56

Analyte	Abbreviation	CAS number	Q1 mass [Da]	Q3 mass [Da]	Retention time [min]
Sodium perfluoro-(1,2,3 – $^{13}C_3$)hexanesulfonate	M3PFHxS		401.90	79.90	5.67
Perfluoro-($^{13}C_8$)octanoic acid	M8PFOA		420.95	376.00	6.35
Perfluoro-($^{13}C_8$)nonanoic acid	M9PFNA		471.94	427.00	7.05
Sodium perfluoro-($^{13}C_8$)octanesulfonate	M8PFOS		506.91	98.90	7.07
Perfluoro-(1,2,3,4,5,6 – $^{13}C_6$)decanoic acid	M6PFDA		518.92	474.00	7.57
N-methyl-d_3 -perfluorooctanesulfonamidoacetic acid	d3-*N*-MeFOSAA		572.89	419.00	7.89
Perfluoro-($^{13}C_8$)octanesulfonamide	M8FOSA		505.98	77.90	7.96
Perfluoro-(1,2,3,4,5,6,7 – $^{13}C_7$)undecanoic acid	M7PFUdA		569.95	525.00	8.03
N-ethyl-d_5 -perfluorooctanesulfonamidoacetic acid	d5-*N*-EtFOSAA		588.85	418.80	8.14
Perfluoro-(1,2 – $^{13}C_2$)dodecanoic acid	MPFDoA		614.95	569.90	8.42
N-methyl-d_3-perfluoro-1-octanesulfonamide	d3-*N*-MeFOSA		515.00	169.00	8.81
N-methyl-d_7-perfluorooctanesulfonamidoethanol	d7-*N*-MeFOSE		623.00	59.00	8.85
Perfluoro-(1,2 – $^{13}C_2$)tetradecanoic acid	M2PFTeDA		714.94	670.00	8.98
N-ethyl-d_5-perfluoro-1-octanesulfonamide	d5-*N*-EtFOSA		531.00	169.00	9.03
N-ethyl-d_9-perfluorooctanesulfonamidoethanol	d9-*N*-EtFOSE		639.00	59.00	9.08
Perfluoro-(2,3,4 – $^{13}C_3$)butanoic acid	M3PFBA		216.00	172.00	1.74
Perfluoro-(1,2 – $^{13}C_2$)hexanoic acid	MPFHxA		315.00	270.00	4.53
Perfluoro-1-hexane($^{18}O_2$)sulfonic acid	MPFHxS		403.00	103.00	5.66
Perfluoro-(1,2,3,4 – $^{13}C_4$)octanoic acid	MPFOA		417.00	372.00	6.35
Perfluoro-(1,2,3,4 – $^{13}C_4$)octanesulfonic acid	MPFOS		503.00	99.00	7.00
Perfluoro-(1,2,3,4,5 – $^{13}C_5$) nonanoic acid	MPFNA		468.00	423.00	7.06
Perfluoro-(1,2 – $^{13}C_2$)decanoic acid	MPFDA		515.00	470.00	7.57

Table 2: MRM transitions and retention times of native PFAS and isotopically labeled PFAS analytical standard

Good to know



Please scan the QR Code for more technical information:

www.mn-net.com/media/pdf/3a/75/c9/Flyer-CHROMABOND-MULTI-96-PFAS_EN.pdf

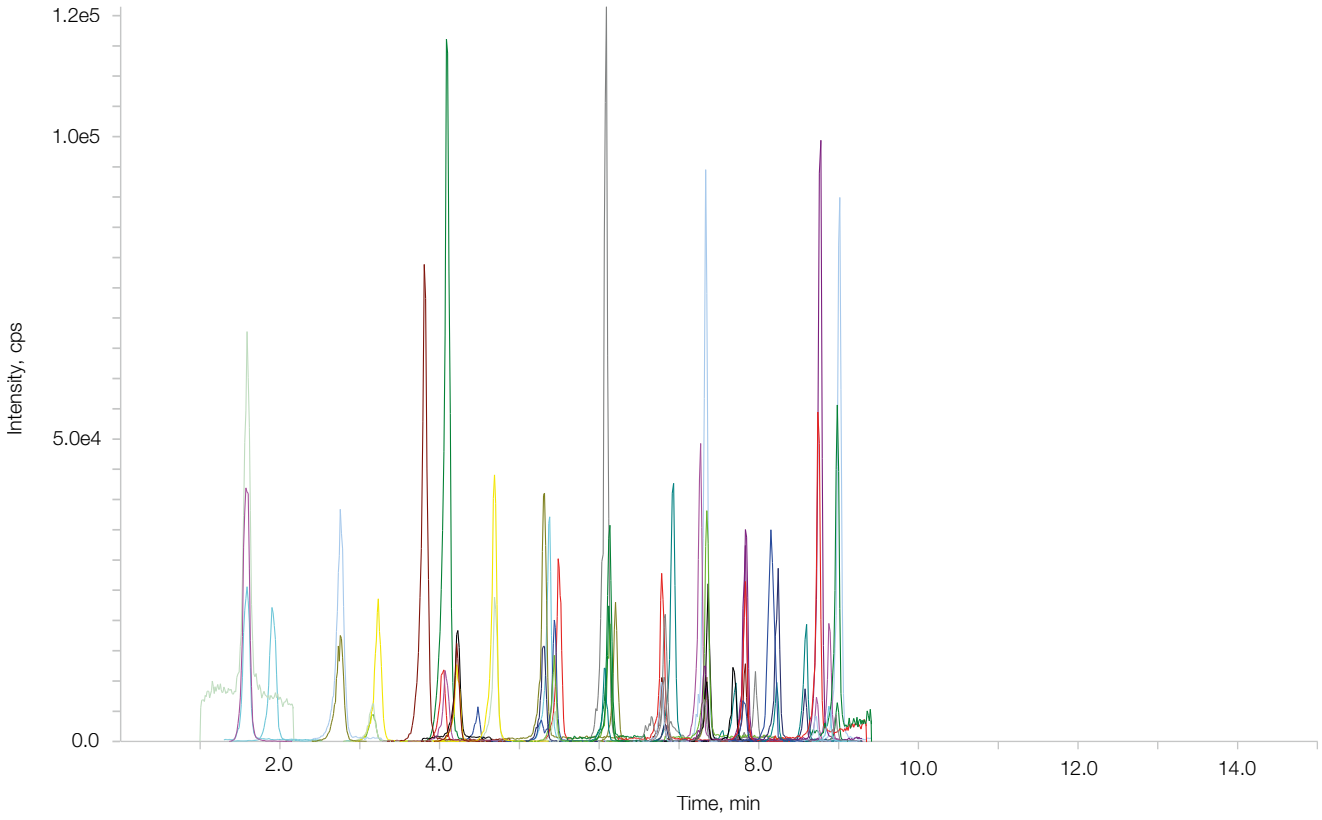


Figure 4: Chromatogram of a standard solution (concentration, β = 1.0 ng/mL)

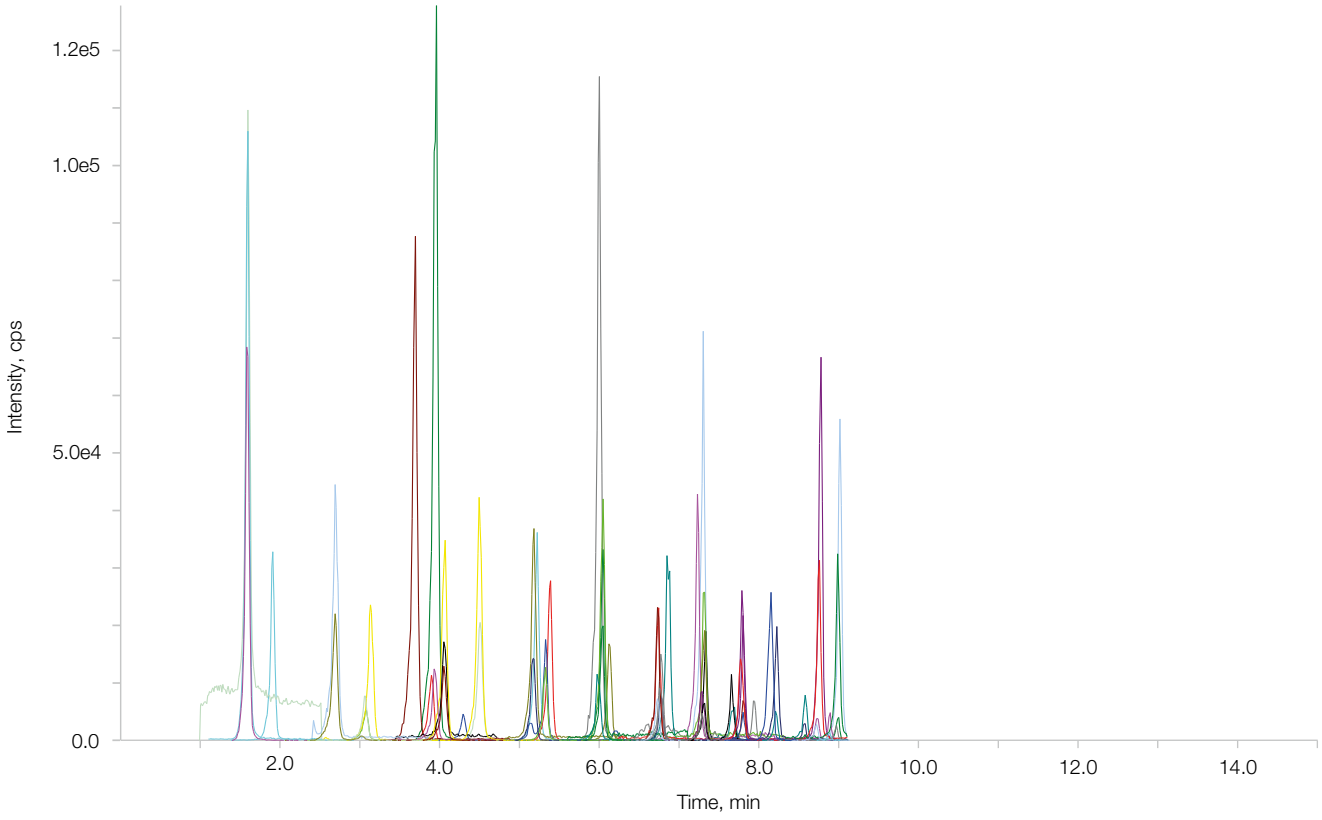


Figure 5: Chromatogram of a solid phase eluate (concentration, β = 1.0 ng/mL)

Results

The combination of Resolvex® A200 and the CHROMABOND® PFAS Multi 96-Monoblock DE enables SPE from human serum for a wide range of different PFAS groups such as carboxylic acids, sulfonic acids, sulfonamides, sulfonamidoacetic acids, chloroperfluoroalkyl ether sulfonates, perfluoroalkyl ether sulfonate per- and polyfluoroalkyl ether carboxylic acids, perfluoroalkane- sulfonamidoethanols, and fluorotelomer carboxylic acids. As shown in Figure 6, most PFAS had recoveries of 90 – 110 %, at a spiking level of 4 ng/mL (for each PFAS analyte) in human serum.

As expected, the recovery rates decrease as the chain length of the analytes increases. The standard deviations, however, increases with chain length of PFAS molecules. It appears that increased surface activity and/or poorer solubility of these non-polar analytes may be a cause for this observation. With the CHROMABOND® PFAS Multi 96-Monoblock DE, built as a dual-layer phase, both ionic and non-ionic PFAS are enriched efficiently. A blank value entry by the Resolvex® A200, through hoses, valves, etc., could not be determined for typical PFAS-concentrations in serum.

As presented in Table 1, the semi-automated workflow requires approximately 20 min to carry out SPE of 96 protein-precipitated serum samples. Systems working with single cartridge workflow would require many times more processing time for the same number of samples. The semi-automated workflow of the Resolvex® A200 positive pressure processor allows users to achieve requested high sample throughput in pharmaceutical diagnostic field.

The chromatographic analysis of SPE eluates on the NUCLEODUR® Phenyl-Hexyl column yielded excellent results. The chromatograms (Figure 4 and 5) show high intensity and good peak shapes for PFAS analytes with low background with a run time of 15 minutes.

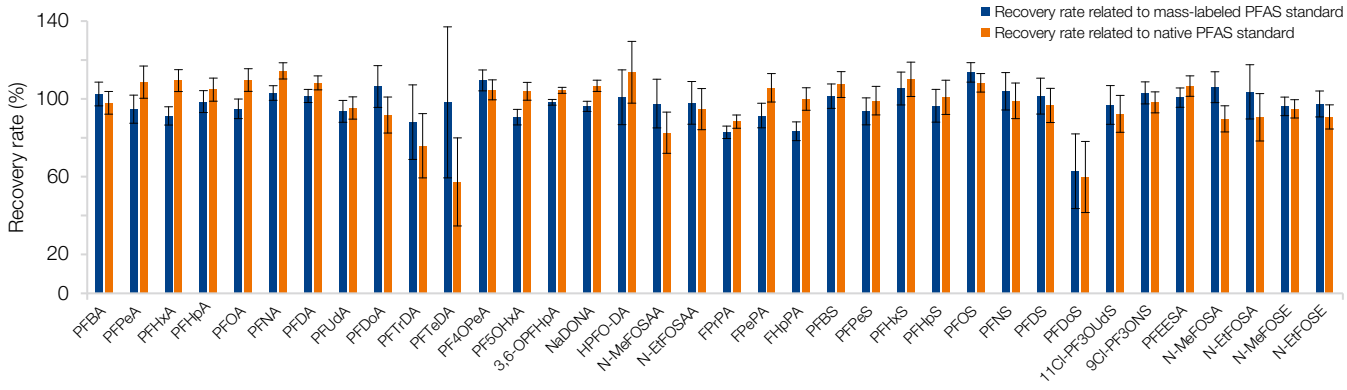


Figure 6: Recovery rates of PFAS from human serum for the presented semi-automated SPE

Analyte (Abbreviation)	Recovery rate (%) related to native PFAS standard	RSD (%) related to native PFAS standard	Recovery rate (%) related to mass-labelled PFAS standard	RSD (%) related to mass-labelled PFAS standrd
PFBA	102.5	6.1	98.0	5.8
PFPeA	94.7	7.2	108.6	8.3
PFHxA	91.3	4.7	109.4	5.6
PFHpA	98.6	5.6	104.8	5.9
PFOA	94.9	5.0	109.7	5.8
PFNA	103.1	3.8	114.4	4.2
PFDA	101.5	3.4	108.3	3.6
PFUdA	93.6	5.6	95.3	5.7
PFDoA	106.4	10.7	91.7	9.3
PFTrDA	88.0	19.2	75.9	16.5
PFTeDA	98.2	38.9	57.3	22.7
PF4OPeA	109.5	5.4	104.7	5.1
PF5OHxA	90.6	4.0	103.9	4.6
3.6-OPFHpA	98.3	1.5	104.4	1.5
NaDONA	96.2	2.6	106.8	2.9
HFPO-DA	100.9	14.1	113.7	15.9
N-MeFOSAA	97.6	12.5	82.6	10.6

Analyte (Abbreviation)	Recovery rate (%) related to native PFAS standard	RSD (%) related to native PFAS standard	Recovery rate (%) related to mass-labelled PFAS standard	RSD (%) related to mass-labelled PFAS standrd
N-EtFOSAA	98.0	11.0	94.7	10.6
FPrPA	82.8	3.2	88.3	3.4
FPePA	91.5	6.3	105.7	7.3
FHpPA	83.4	4.8	100.0	5.8
PFBS	101.4	6.3	107.4	6.6
PFPeS	93.6	6.9	99.1	7.3
PFHxS	105.4	8.5	110.1	8.8
PFHpS	96.5	8.4	100.8	8.8
PFOS	113.7	5.0	108.3	4.8
PFNS	103.9	9.6	99.0	9.2
PFDS	101.5	9.2	96.7	8.8
PFDoS	62.8	19.2	59.8	18.3
11Cl-PF3OUdS	96.9	10.0	92.3	9.5
9Cl-PF3ONS	103.1	5.7	98.2	5.4
PFEESA	100.7	5.0	106.6	5.3
N-MeFOSA	106.0	7.9	89.7	6.7
N-EtFOSA	103.6	14.0	90.5	12.2
N-MeFOSE	96.2	4.8	94.9	4.7
N-EtFOSE	97.4	6.7	90.7	6.2

Table 3: Recovery rates of PFAS from human serum for the presented semi-automated SPE method using CHROMABOND® Multi 96 PFAS plate on Resolvex® A200 (Tecan, Männedorf, Switzerland).

Summery

This application note presents the reliable and successful determination of 36 PFAS from human serum. By using the Resolvex® A200 positive pressure processor (Tecan, Männedorf, Switzerland) in combination with a dual-layer CHROMABOND® Multi 96 PFAS Monoblock DE plate, it was possible to achieve high recovery rates with good reproducibility for various PFAS groups. The semi-automated SPE workflow requires approximately 20 min to carry out SPE of 96 protein-precipitated serum samples and allows users to achieve requested high sample throughput in pharmaceutical diagnostic field. The identification and the quantification of PFAS in sample extracts were finally carried out by ESI mass spectrometry on a NUCLEODUR® Phenyl-Hexyl column with a run time of 15 minutes.

Literature

[1] Nicole M. DeLuca, Jeffrey M. Minucci, Ashley Mullikin, Rachel Slover, Elaine A. Cohen Hubal, Human exposure pathways to poly- and perfluoroalkyl substances (PFAS) from indoor media: A systematic review, Environment International, Volume 162, April 2022, 107149, *https://doi.org/10.1016/j.envint.2022.107149*

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[3] Zeng Z, Song B, Xiao R, Zeng G, Gong J, Chen M, Xu P, Zhang P, Shen M, Yi H. Assessing the Human Health Risks of Perfluorooctane Sulfonate by In Vivo and In Vitro Studies. Environment International. 2019 126:598–610. doi: *https://doi.org/10.1016/j.envint.2019.03.002.*

[4] United States Environmental Protection Agency. Health Effects Support Document for Perfluorooctane Sulfonate (PFOS). USEPA 2016; EPA 822-R-16–002.

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