Application

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PFAS's by Automated mixed-mode µSPEed® cartridges on ePrep® Environmental Application - Contaminated Surface Water

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INTRODUCTION

Perfluoroalkyl substances (PFAS) are a family of synthetic fluorine-containing chemicals that consist of a fluorinated hydrocarbon chain bonded to a charged group. PFAS has many desirable chemical properties for commercial and industrial applications, such as protective coatings for fabrics, manufacture of surfaces for non-stick cookware and flame retardants in fire-fighting foams.

PFAS are ubiquitous and persistent and contaminate soil and water resources, the local environment, and are detected across the entire earth, even in very remote areas. Animal studies, primarily performed in rodents, have shown links between perfluoro-octane sulfonate (PFOS) and perfluoro-octanoic acid (PFOA) exposure and increased liver weight, behavioural and developmental changes in offspring, negative reproductive effects and tumour growth. Large scale studies of human exposure have also linked PFOS and PFOA to high cholesterol, thyroid disease, autoimmune disease, and testicular and kidney cancer.

Food Standards Australia New Zealand (FSANZ) recommends that drinking water should contain less than 70 and 560 ng L^{-1} (parts per trillion) for PFOS and PFOA, respectively[1]. Solid phase extraction (SPE) is the preferred method[2] for the clean-up and pre-concentration of PFAS contaminated water samples. While effective, SPE is labour and time-intensive and requires large sample volumes to achieve the regulatory limits of detection specified by FSANZ.

The introduction of ePrep's Sample Preparation Workstation offers an innovative alternative to traditional SPE techniques, eliminating labour intensive processes to increase precision and accuracy vastly. ePrep has been designed for automated standard and sample dilution, and micro solid phase extraction (µSPE). The high pressure µSPEed Cartridges is a significant advancement over current SPE cartridges as 3µm sorbents are packed into an 8µl (4.2mg) bed volume,

Figure 1: ePrep Sample Preparation Workstation and µSPEed cartridges with one-way check valves.

providing enormous separation power and high concentration factors in µl volumes.

This application note demonstrates the utility of the Sample Preparation Workstation for the microSPE sample preparation of perfluoroalkyl carboxylates and sulfonates extracted from contaminated surface water using mixed-mode PFAS µSPEed cartridges ***in less than 5 min***[3].

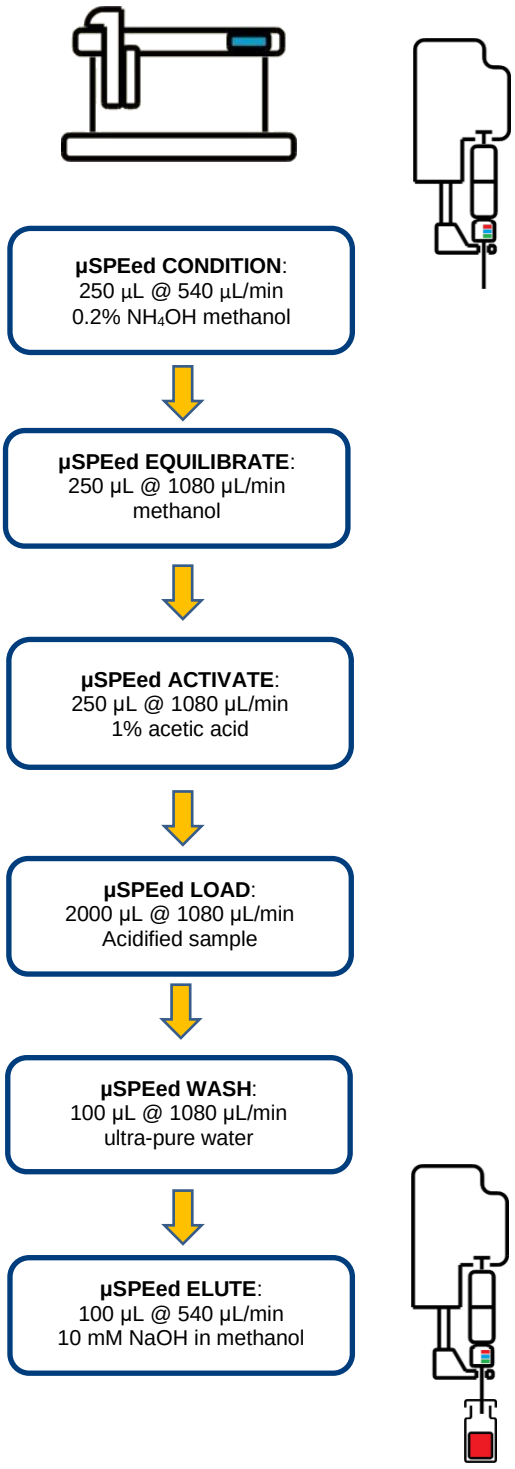

Figure 2: Sample clean-up and pre-concentration

METHODS

The µSPEed is a unique design of SPE cartridge for automated sample preparation in conjunction with the ePrep Sample Preparation Workstation (Figure 1). The typical operation involves loading a sample from a designated vial by drawing the sample into the syringe through the one-way check valve. The sample is loaded by reversing the direction of flow by depression of the syringe, where the check valve is closed, directing the sample to flow through the sorbent. The sample is eluted with an eluting solvent by repeating this process.

Solutions:

- 0.2% NH_4OH in methanol
- Methanol
- 1% acetic acid
- ultra-pure water
- 0.1% acetic acid

Sample Preparation

Before extraction 10 mL aliquots of samples were acidified to approximately pH 3 with 100 µL of glacial acetic acid to aid in the retention of short-chain PFASs. Samples were spiked with 100 ng L^{-1} of the internal standard mixture.

Figure 2 details the clean-up and pre-concentration of samples using ePrep/µSPEed before chromatography. The eluent was diluted with 100 µL of 0.1% acetic acid to improve peak shape.

UHPLC

Gradient separation of the 13 PFAS compounds was performed on a Shimadzu Nexera X2 using a Shimadzu Shim-pack XR-ODSIII 2.0 × 50 mm, 1.6 µm column.

Conditions:

Column: Phenomonex Luna Omega 2.1 x 50 mm, 1.6 µm C18
Isolator Column: ACQUITY® PFC isolator column installed between the solvent mixer and autosampler to mitigate PFAS contamination.
Flowrate: 0.4 ml $min^{-1.}$
Injection: 40µL
Column Temp: Isothermal at 40 °C.

Mobile phase:

Mobile Phase A: ultra-pure water with 2 mM ammonium acetate
Mobile Phase B: methanol with 2 mM ammonium acetate.

Gradient

Initial: 80% A / 20% B held for 0.2 min
Gradient 1: change to 30% A / 70% B over 2.4 min
Gradient 2: change to 5% A / 95% B over 5 min.
Final: 5% A / 95% B held for 2 min
Return to the initial conditions and equilibrated for 4 min.

MS/MS

Detection was performed using a Shimadzu LCMS-8060 triple quadrupole mass spectrometer operated in negative ionisation mode. The interface voltage and temperature were optimised to -0.5 kV and 300 °C. Nebulising, heating and drying gas flows were set at 3, 10 and 10 L min^{-1} respectively and collision gas was operated at a pressure of 270 kPa. Multiple reaction monitoring (MRM) mode using the parameters in Table 1 was used and optimised for transitions from the $[M-H]^-$ ion using the LabSolutions software. Where possible, both a quantification and identification ion were used for each compound however some compounds, such as PFBA, exhibit only one significant fragmentation. (Figure 3)

Table 1. Multiple reaction monitoring parameters used during analysis.

Compound	Abbreviation	Transition (m/z)	Q1 PreBias (V)	Collision Energy (eV)	Q3 PreBias (V)
Perfluorobutanoic acid	PFBA*	213.05 → 169.00	5	9	9
Perfluoropentanoic acid	PFPeA*	262.95 → 218.95	9	8	29
Perfluorohexanoic acid	PFHxA*	312.95 → 268.90	7	9	35
	PFHxA	312.95 → 118.90	11	19	19
Perfluoroheptanoic acid	PFHpA*	362.95 → 319.00	13	10	15
	PFHpA	362.95 → 169.05	13	17	9
Perfluorooctanoic acid	PFOA*	412.95 → 369.00	15	10	11
	PFOA	412.95 → 169.05	15	17	9

Perfluorononanoic acid	PFNA*	462.95 → 419.00	11	11	13
	PFNA	462.95 → 219.00	11	17	13
Perfluorodecanoic acid	PFDA*	512.90 → 468.95	19	11	15
	PFDA	512.90 → 268.90	19	17	13
Perfluoroundecanoic acid	PFUnA*	562.90 → 518.85	13	11	13
	PFUnA	562.90 → 319.10	9	19	21
Perfluorododecanoic acid	PFDoA*	612.90 → 568.95	15	12	11
	PFDoA	612.90 → 319.15	15	18	9
Perfluorotetradecanoic acid	PFTeDA*	712.90 → 668.90	17	13	17
	PFTeDA	712.90 → 168.85	19	26	27
Perfluorobutane sulfonate	PFBS*	298.90 → 80.00	11	32	11
	PFBS	298.90 → 99.00	11	26	5
Perfluorohexane sulfonate	PFHxS*	399.00 → 80.00	11	40	9
	PFHxS	399.00 → 99.05	13	34	5
Perfluorooctane sulfonate	PFOS*	499.00 → 80.00	9	58	9
	PFOS	499.00 → 99.05	9	40	5

* Quantification ion.

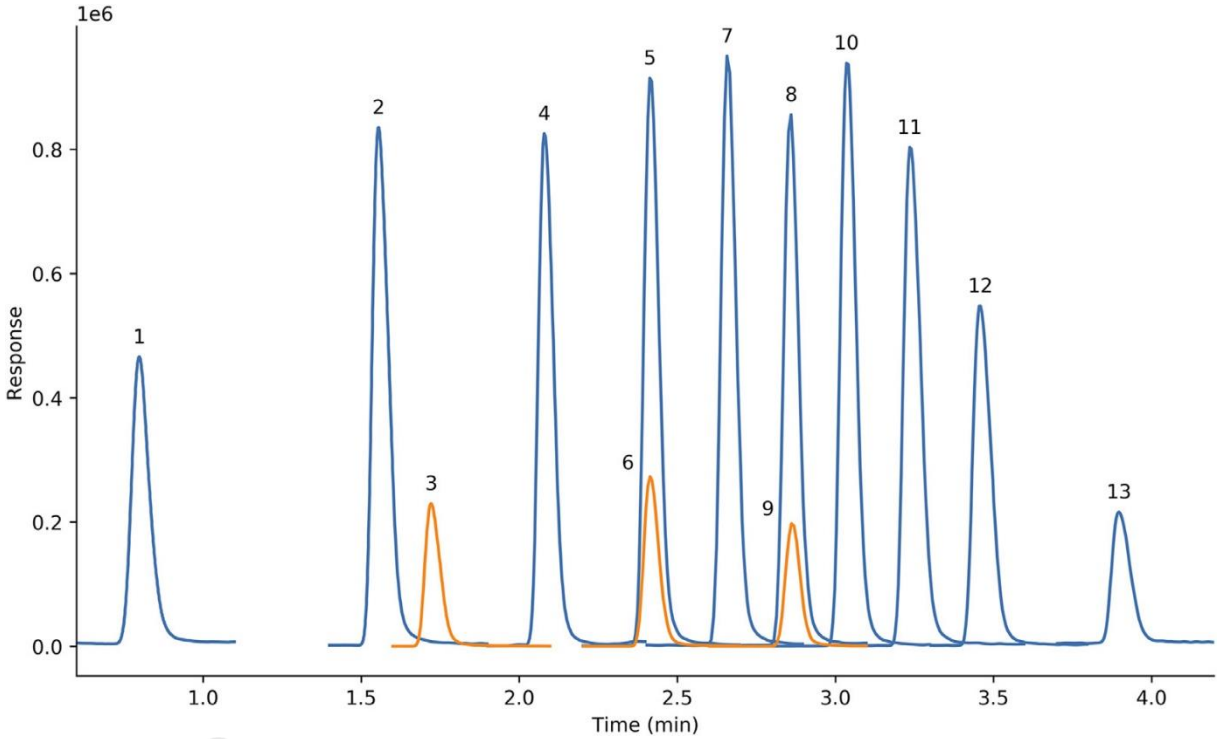

Figure 3 UHPLC separation of the perfluoroalkyl carboxylates (blue) and sulfonates (orange): PFBA (1), PFPeA (2), PFBS (3), PFHxA (4), PFHpA (5), PFHxS (6), PFOA (7), PFNA (8), PFOS (9), PFDA (10), PFUnA (11), PFDoA (12), PFTeDA (13).

RESULTS

A seven-point calibration curve ranging from 10 to 9000 ng L^{-1} was constructed and used to determine the linearity and instrumental limits of detection (Table 2). Recoveries of internal standards were also determined (Table 3).

Recoveries of the PFAS were calculated to be between 86 and 111% across the sample range. Low standard deviations highlighted the reproducibility of µSPEed for the extraction of PFASs from surface waters with LODs well below the regulatory limits.

Table 2. Validation results for the extraction and instrument methods (n=6).

Compound	%Recovery	%RSD	Linearity	LOD (ng L^{-1})
PFBA	101	15	0.9998	3.5
PFPeA	98	10	0.9998	2.8
PFHxA	101	12	0.9999	5.2
PFHpA	106	3	0.9999	1.2
PFOA	97	8	0.9998	1.7
PFNA	107	7	0.9998	2.2
PFDA	96	18	0.9997	1.7
PFUnA	104	12	0.9995	4.4
PFDoA	103	3	0.9993	4.8
PFTeDA	86	6	0.9992	6.6
PFBS	105	4	0.9998	0.29
PFHxS	105	12	0.9997	1.3
PFOS	111	12	0.9995	2.7

Table 3. Recoveries of the isotopically enriched PFAS internal standards from spiked water. Results are reported as the mean recovery and standard deviation (n = 3).

Internal Standard	%Recovery	%RSD
[$^{13}C_4$]PFBA	**50**	**3**
[$^{13}C_5$]PFPeA	110	1
[1,2,3,4,6- $^{13}C_5$]PFHxA	123	1
[1,2,3,4- $^{13}C_4$]PFHpA	121	1
[$^{13}C_8$]PFOA	107	1
[$^{13}C_9$]PFNA	95	2
[1,2,3,4,5,6- $^{13}C_6$]PFDA	75	3
[1,2,3,4,5,6,7-$^{13}C_7$]PFUnA	50	1
[1,2- $^{13}C_2$]PFDoA	55	2
[1,2- ^{13}C2]PFTeDA	65	5
[2,3,4- $^{13}C_3$]PFBS	102	3
[1,2,3- $^{13}C_3$]PFHxS	110	4
[$^{13}C_8$]PFOS	88	4

The extraction method was validated using spiked recoveries from the PFAS contaminated water samples. Six unique samples of differing PFAS concentration and salinity were spiked (100 ng L^{-1} PFAS), extracted in triplicate and quantified using internal standard calibrations. The percent recovery and standard deviation for each compound over the six samples was then determined (Table 4)

PFAS was detected in all six of the samples, with concentrations ranging from <LOD to 898 ± 15 ng L^{-1}. Recoveries of spikes were between 95 and 110% for most compounds with %RSDs below 20%

Table 4. Quantities of PFAS detected in the six surface water samples. Results are reported as the mean ± SD in ng L^{-1} (n = 3).

	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnA	PFDoA	PFTeDA	PFBS	PFHxS	PFOS
A	340±40	350.2±1.1	376.8±1.5	124.2±1.4	39.9±0.4	5.7±0.7	4.3±1.1	15±7	ND	40±20	76.4±0.2	898±15	860±30
B	610±30	11.6±0.3	48±2	3.5±0.4	2.5±0.2	6±3	7.1±1.6	25±12	20±13	50±40	66±2	254±3	10.6±0.7
C	800±300	5.3±0.3	9.8±0.4	2.2±0.2	2.4±0.2	3.8±0.4	3.5±0.3	12.6±1.3	ND	ND	3.5±0.5	3.9±0.3	4.9±0.9
D	225±23	33.4±1.0	76.4±1.0	31.40±0.13	65.6±1.4	8.6±1.0	7.3±0.5	23.9±1.6	15.6±1.4	30±12	20.49±0.16	88.4±1.1	25.5±1.0
E	40±6	8.9±0.2	29.2±0.7	2.70±0.03	5.3±0.5	1.6±0.6	4.4±1.5	ND	ND	ND	61.8±1.2	198±6	582±13
F	300±130	ND	5.1±0.3	ND	1.52±0.02	1.8±1.3	2.2±0.6	ND	ND	ND	7.4±0.4	7.1±1.1	ND

ND = Not detected.

CONCLUSIONS

µSPEed-PFAS microSPE cartridges are an alternative to conventional SPE for the extraction of C_{4-10} poly and perfluoroalkyl acids.

The instrument detection limits for compounds tested were less than 10 ng L^{-1}. The lowest Australian guidance value for PFASs in water is 70 ng L^{-1} (PFOS), making this method suitable for monitoring environmental waters with no additional clean-up required.

A typical extraction took 5 minutes to perform manually, faster than conventional SPE methods such as EPA Method 537 (>30 minutes). µSPEed achieved similar results using a 125 x smaller sample volume (2 mL vs 250 mL) than the conventional SPE methods. It is also noted that conventional SPE methods use larger volumes of elution solvent do obtain a higher level of pre-concentration achieved by evaporation and reconstitution of the extract (8 ml evaporated to ~0.5 mL and reconstituted in 1 mL for EPA Method 537), a process that can take several hours and may potentially lead to loss of more volatile PFASs such as fluorotelomer alcohols.

Note: Contamination is a common issue that arises during the analysis of PFASs as they are commonly used as polymerisation aids in the manufacture of polytetrafluoroethylene (PTFE) and other fluoropolymers. Fluoropolymers should be avoided as much as possible. In this study PFAS-free polypropylene and polyethylene containers, lids and vial septa were used for all reagents, standards and samples.

ACKNOWLEDGEMENTS

Lockwood, T.; Bishop, D; Maleknia, S; Doble, P.
University of Technology Sydney - School of Mathematical and Physical Sciences, Faculty of Science.

REFERENCES

[1] Perfluorinated Chemicals in Food; Food standards Australia New Zealand; 2017.

[2] Shoemaker, J. A.; Grimmett, P. E.; Boutin, B. K. METHOD 537; 2009.

[3] Lockwood, T.; Bishop, D.; Maleknia, S.; Minett, A.; Dawes, P.; Doble, P. Automated µSPE for the determination of PFAS compounds, Proceedings of the 66th American Society for Mass Spectrometry Conference on Mass Spectrometry and Allied Topics, June 3-7, San Diego, USA; 2018.

POSTSCRIPT

PFAS-75 (75% WAX and 25% C18) was trialled to improve speed of elution. The hypothesis was that the higher WAX would reduce breakthrough of low Carbon analytes. Figure 4a and b indicated this to be the case.

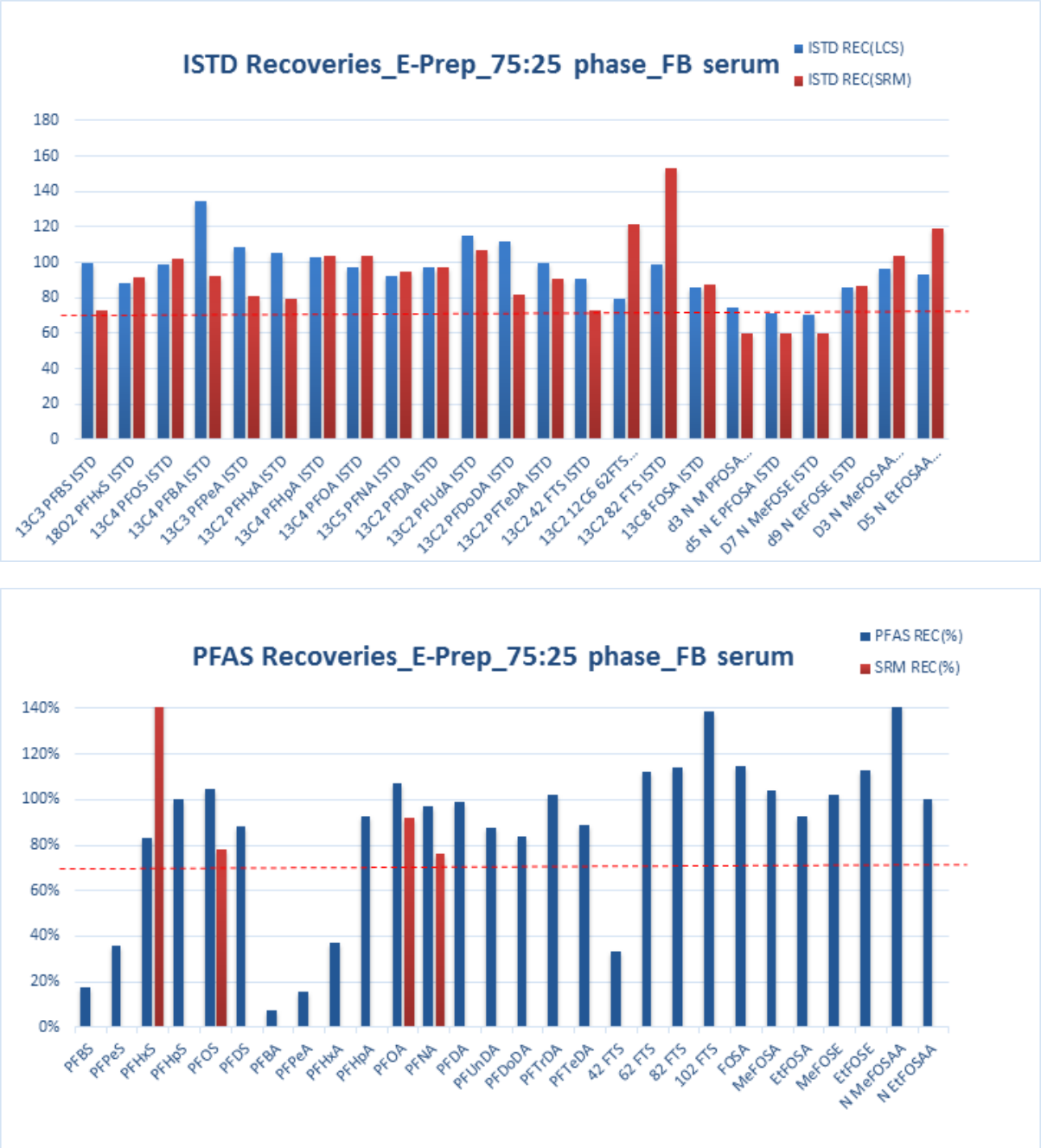

Figure 4a & b ISTD Recoveries show excellent results. PFAS recoveries were good but could be improved through better matrix tuning to reduce signal suppression.

µSPEed Cartridge Ordering Information

Part No	Description
01-10105	C4, 3 µm / 120Å Silica µSPEed Cartridges (Pkt 10)
01-10106	C8, 3 µm / 120Å Silica µSPEed Cartridges (Pkt 10)
01-10110	C18 (ODS), 3 µm / 120Å Silica µSPEed Cartridges (Pkt 10)
01-10111	C18 (ODS), 3 µm / 120Å Silica µSPEed Cartridges (Pkt 50)
01-10115	Silica, 3 µm / 120Å µSPEed Cartridges (Pkt 10)
01-10116	Silica, 3 µm / 120Å µSPEed Cartridges (Pkt 50)
01-10117	WAX (APS), 3 µm / 120Å µSPEed Cartridges (Pkt 10)
01-10118	PFAS-50 {50% WAX}, 3 µm / 120Å µSPEed Cartridges (Pkt 10)
01-10119	PFAS-75, {75% WAX} 3 µm / 120Å µSPEed Cartridges (Pkt 10)
01-10124	C18/P Hydrophilic ODS 3 µm / 120Å µSPEed Cartridges (Pkt 10)
01-10135	µCARB, 3µm/250Å µSPEed cartridges (Pkt 10)
01-10149	PS/DVB, 3 µm / 300Å µSPEed Cartridges (Pkt 50)
01-10150	PS/DVB, 3 µm / 300Å µSPEed Cartridges (Pkt 10)
01-10151	PS/DVB-Phenyl, 3 µm / 300Å µSPEed Cartridges (Pkt 10)
01-10155	SAX-PS/DVB, 3 µm / Non-Porous µSPEed Cartridges (Pkt 10)
01-10156	SCX-PS/DVB, 3 µm / Non-Porous µSPEed Cartridges (Pkt 10)
01-10185	Cxyl 3 µm, Customisable Chemistry µSPEed Cartridges (Pkt 10)