TN-0146

Analysis of PFAS in Drinking Water by EPA Method 537.1: A Direct Comparison of the Accuracy and Precision of Manual and Automated SPE Sample Preparation

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Introduction

Method 537.1 is a solid phase extraction (SPE) liquid chromatography in tandem with mass spectrometry (LC-MS/MS) method for the determination of selected per- and polyfluorinated alkyl substances (PFAS) in drinking water. Method 537.1 will be part of the upcoming UCMR5, with a focus on PFAS. A critical part of the method is the SPE sample preparation-concentration step which employs a Styrene-DVB copolymer in tube format. Proper performance of this sample preparation step is essential for producing accurate and precise laboratory results. Laboratories can use either a manual or an automated SPE procedure. Therefore, laboratories trying to decide which procedure to deploy may wish to know how well the two techniques perform regarding the expected accuracy and precision of analytical results. In this technical note, we enlisted the assistance of the Orange County Water District (OCWD), a well-regarded drinking water laboratory highly experienced in the application of EPA Method 537.1, to make a direct comparison of the two techniques.

Automated SPE Protocol

Instrumentation:	PromoChrom Technologies SPE-03 Automated SPE System with MOD-004 (sample bottle rinsing) and MOD-005 (minimal Teflon option).	
Sample Volume:	250 mL	
SPE Cartridge:	Strata® SDB-L; 500 mg/6 mL (Part No. 8B-S014-HCH)	
Solvent 1:	Methanol	
Solvent 2:	Milli-Q® water	
Sample Extraction:	See Table 1.	
Extract Concentration:	Concentration Settings: Temperature = 55 °C, Pressure = 10 psi	
	Concentrate:	Eluate to dryness and allow to cool for 1 minute.
	Add:	1 mL of methanol/water (96:4, v/v) to the concentration tube with a micro pipette.
	Add:	10 µL of IS-working standard and apply a gentle vortex.
	Transfer:	To three polypropylene vials with starburst caps.
	Store:	Remaining extracts in centrifuge tubes.

Manual SPE Protocol

Instrumentation:	Vac Elut™ 20 vacuum extraction manifold with vacuum pump.	
SPE Cartridge:	Strata SDB-L; 500 mg/6 mL (Part No. 8B-S014-HCH)	
Cartridge Clean Up and Conditioning:	**Rinse:**	Cartridge with 3 aliquots of 5 mL methanol.
	Rinse:	Cartridge with 3 aliquots of 6 mL Milli-Q water.
	Add:	4 mL Milli-Q water to the top of the cartridge.
Sample Extraction:	**Fill:**	Reservoirs with sample before turning on vacuum.
	Adjust:	Pressure to give a 10-15 mL/min flow rate (25-30 min/sample).
	Stop:	Flow with about 10 mL remaining in the reservoirs and rinse the sample bottle with 8 mL Milli-Q water and add to reservoirs. Repeat this step again.
	Pull:	Air through cartridge for 10 min at high vacuum (10-15 in. Hg).
Cartridge Elution:	**Turn Off:**	And release vacuum.
	Place:	Collection tubes into the extraction manifold.
	Rinse:	Sample bottle with 4 mL methanol (swirl down the sides of the reservoir).
	Reduce:	The vacuum power (5 mL/min) so the solvent exits the cartridge in a slow, drop-wise fashion. Do NOT completely dry the cartridge.
	Repeat:	Elution step, but dry cartridge completely.
	Transfer:	Eluents to concentration tubes.
	Rinse:	Collection tubes with 3 mL methanol and add to the concentration tubes.
	Repeat:	Prior rinse again.
Extract Concentration:	Concentration Settings: Temperature = 55 °C, Pressure = 10 psi	
	Concentrate:	Eluate to dryness and allow to cool for 1 minute.
	Add:	1 mL of methanol/water (96:4, v/v) to the concentration tube with a micro pipette.
	Add:	10 µL of IS-working standard and apply a gentle vortex.
	Transfer:	To three polypropylene vials with starburst caps.
	Store:	Remaining extracts in centrifuge tubes.

Table 1. PromoChrom SPE-03 Extraction Procedure

Step	Action	Inlet	Flow Rate (mL/min)	Volume (mL)	Time (min)
1	Elute W2	Solvent 1	5	5	-
2	Wait	-	-	-	1
3	Elute W2	Solvent 1	3	5	-
4	Wait	-	-	-	1
5	Elute W2	Solvent 1	3	5	-
6	Wait	-	-	-	2
7	Elute W1	Solvent 2	5	18	-
8	Wait	-	-	-	1
9	Elute W1	Solvent 2	5	5	-
10	Wait	-	-	-	2
11	Add Sample W1	Sample	10	285	-
12	Rinse W1	Solvent 2	10	7.5	-
13	Rinse W1	Solvent 2	10	7.5	-
14	Add Sample W1	Sample	10	4.5	-
15	Elute W1	Solvent 1	10	0.2	-
16	Air-Purge1	Air	10	5	-
17	Blow N_2				5 @ 2.0 L/min
18	Rinse 1	Solvent 1	5	4	-
19	Wait	-	-	-	2
20	Rinse 1	Solvent 1	5	4	-
21	Wait	-	-	-	2
22	Collect 1	Sample	5	4.5	-
23	Air-Purge1	Air	5	10	-

LC Conditions

Column: Gemini® 3 µm C18, 100 x 2.0 mm (00D-4439-B0)
Luna® 5 µm C18(2), 30 x 2.0 mm (00A-4252-B0)

Mobile Phase: A: 20 mM Ammonium acetate in water
B: Methanol

Gradient:

Time (min)	%B
0	5
0.1	55
5.4	99
9	99
10	99
10.1	5
12	5

Flow Rate: 430 µL/min
Injection Volume: 5 µL
Temperature: 40 °C
LC System: Agilent® 1260 Infinity
Detection: LC-MS/MS
Detector: 6500+ QTRAP® (SCIEX®)

MS Conditions

Ion Source: Negative
Source Temperature: 400 °C
GS1: 40
GS2: 40
CUR: 35
IS: -4500

Table 2. MRM Transitions

Analyte	Q1 (m/z)	Q2 (m/z)
PFBS	298.9	79.9
PFHxA	312.9	268.9
HFPODA	284.8	168.9
PFHpA	362.9	318.9
PFHxS	398.9	79.9
ADONA	376.9	250.9
PFOA	412.9	368.8
PFOS	498.9	79.9
PFNA	462.9	418.9
9CLPF3	530.8	350.9
PFDA	512.9	468.9
MeFOSA	569.9	418.9
PFUnA	562.9	518.9
EtFOSA	583.9	418.9
11CLPF	630.8	450.9
PFDoA	612.8	568.9
PFTrDA	662.9	618.9
PFTA	712.9	668.9

Results and Discussion

Figure 1 shows a chromatogram for a 2 ng/L calibration standard demonstrating excellent separation of the EPA 537.1 analyte panel. **Figure 2** shows a chromatogram of a 2 ng/L Laboratory Fortified Blank (LFB) showing excellent resolution of all analytes near the lower end of the quantitation range. **Figures 3-5** present the mean recoveries of 18 PFAS analytes that were spiked into reagent water at levels of 50, 20, and 2 ng/L respectively. The 18 analytes were selected from the suite of 27 analytes to show a broad diversity of compound chemical class and properties. For direct comparison, each analyte is displayed in two color-coded recovery bars. The blue bar designates mean analyte % recovery for the manual extraction process and the red bar designates mean analyte % recovery for the automated SPE-03 extraction process. In **Table 3,** the recovery data from **Figures 3-5** are summarized in tabular form to show the overall comparison of the two techniques at the three spiking levels. The averaged summary of the mean recovery data is presented in **Table 4**.

The recovery data presented here shows all analytes falling within the acceptable recovery range of >50 % to <150 % for 2 ng/L and >70 % to <130 % for 20 ng/L and 50 ng/L. A casual visual examination of **Figures 3-5** also suggests a high degree of consistency between the manual and automated sample preparation approaches with minor, random variations between the two techniques over the full range of concentrations. These observations are confirmed by the tabulated values in **Table 3** and by the average recoveries and deviations in **Table 4**.

As seen in **Table 4** there are some minor variations between the two techniques, but these differences are more suggestive than prescriptive. The averages of the standard deviations for both techniques are well below 10 % and are essentially equal. However, the average deviation of the manual technique is slightly more consistent over the full concentration range, whereas the automated technique shows slightly higher (but acceptable) variation at the lower end of the concentration range.

The OCWD has extensive experience in the application of EPA Method 537.1 and estimates that they have analyzed between 5,600 and 6,000 PFAS drinking water samples by EPA Method 537, 537 Version 1.1 and 537.1 since the inception of the 537 method and the UCMR3 program. With the current heightened focus on PFAS in drinking water, they are currently analyzing between 1,800 and 2,000 PFAS sample per year, a rate that is certain to increase with the advent of UCMR5.

OCWD's experience with EPA Method 537.1 in a production laboratory setting reveals some interesting contrasts between the economics of manual and automated PFAS sample preparation. The manual preparation allows for 30 sample bottles (total of field and QC samples) to be processed by one technician in a 9 - 10-hour shift. The automated preparation, however, allows for 48 sample bottles (total of field and QC samples) to be processed by one technician in a 9 – 10-hour shift.

These averages suggest that the automated procedure has a 60% higher throughput (per technician, per shift) than that of the manual method, resulting in higher laboratory labor productivity as well as higher sample throughput. In practice, however, the laboratory labor productivity gains can be significantly higher than 60 % because the automated method – once set up and running – allows "walk-away" operation, permitting the technician to go and perform other laboratory duties while the batch of samples is running. In contrast, the manual method, while not always requiring complete analyst attention, does require frequent input and adjustment which restricts the ability of the technician to be absent for longer periods of time. In fact, the greatest quality risk when conducting Method 537.1 manual sample preparation is to allow the SPE column to go dry. Therefore, the technician's time must be closely focused on maintaining SPE batch process integrity.

Of course, the primary barrier to the automation of sample preparation is the high capital cost of the instrumentation which runs in the tens of thousands of dollars, nearly 10X higher than a manual SPE manifold and vacuum pump. At a certain level of sample throughput, the total cost of the automated method (including capital depreciation) becomes lower than that of the manual method on a total $/sample basis. (Note that the consumable consumption costs of the two approaches are virtually identical on a $/sample basis.) Therefore, high throughput laboratories tend to migrate to the automated solution and lower throughput laboratories are comfortable staying with the manual method. Each laboratory must make an analysis and buying decision based upon their specific circumstances. However, in either case, the laboratory can be assured that, whether manual or automated, the Strata® SDB-L SPE cartridges, and Gemini® C18 and Luna® C18(2) HPLC columns will provide accurate and precise analytical data.

Figure 1. HPLC Chromatogram of a 2 ng/L Calibration Standard

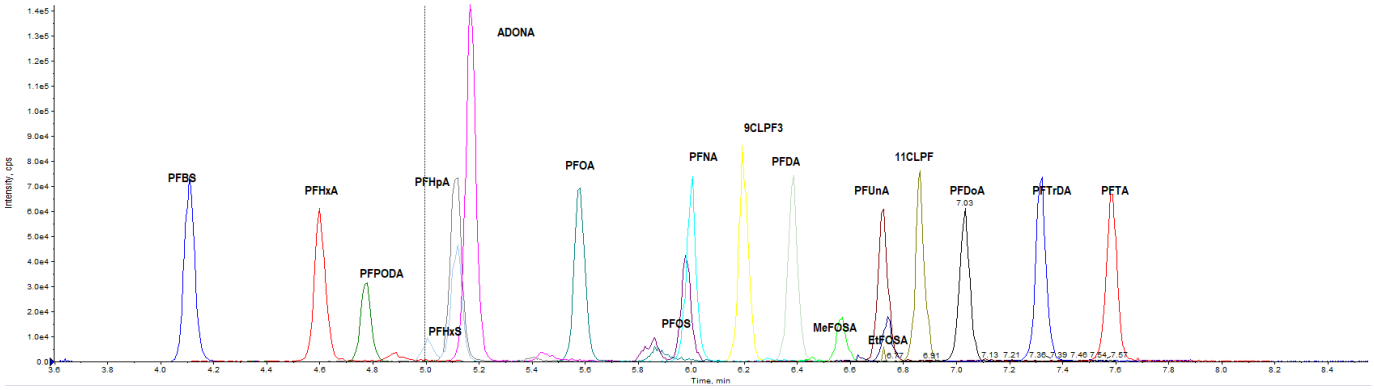

Figure 2. HPLC Chromatogram of a 2 ng/L LFB

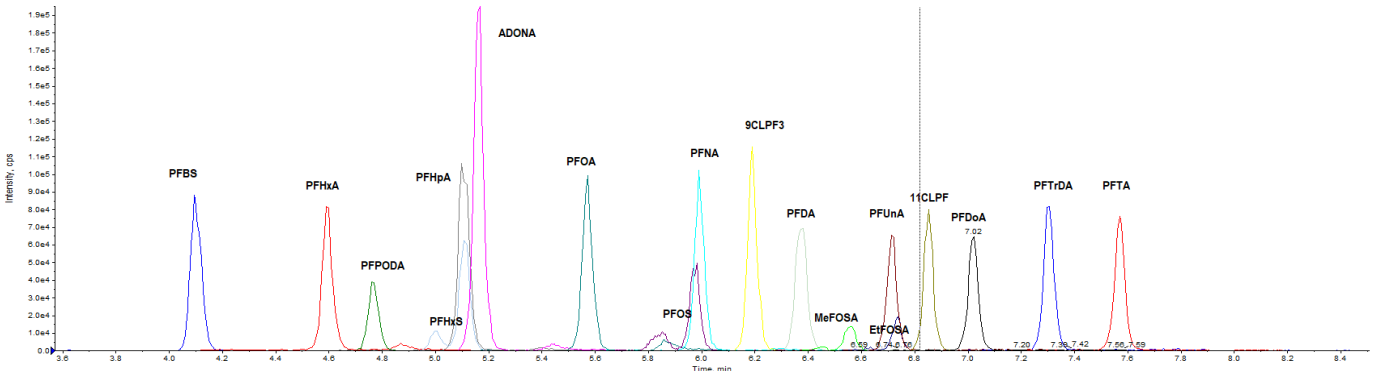

Figure 3. Comparison of Mean Analyte % Recoveries from 50 ng/L LFB, n=19

Figure 4. Comparison of Mean Analyte % Recoveries from 20 ng/L LFB, n=19

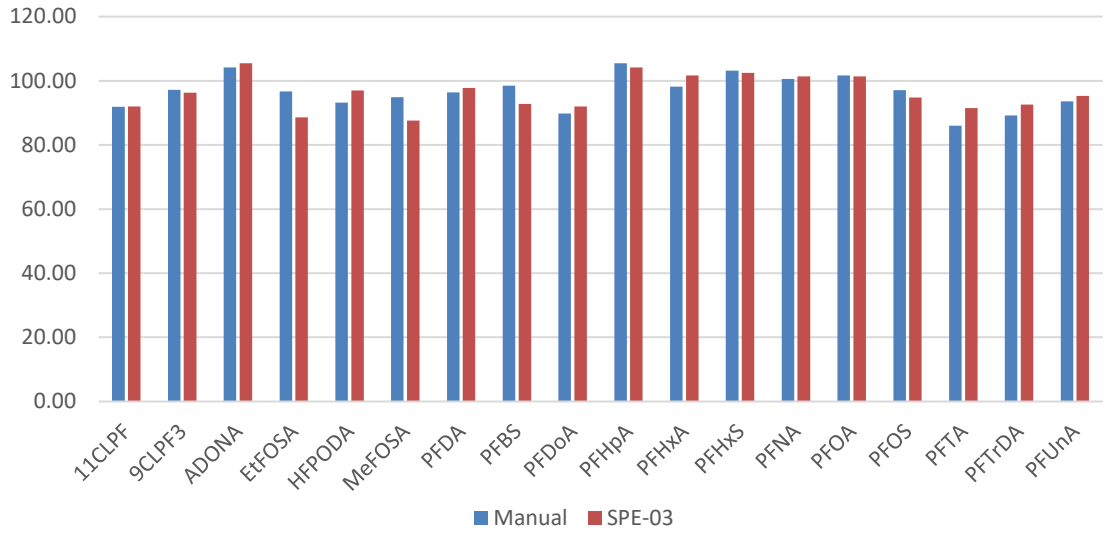

Figure 5. Comparison of Mean Analyte % Recoveries from 2 ng/L LFB, n=19

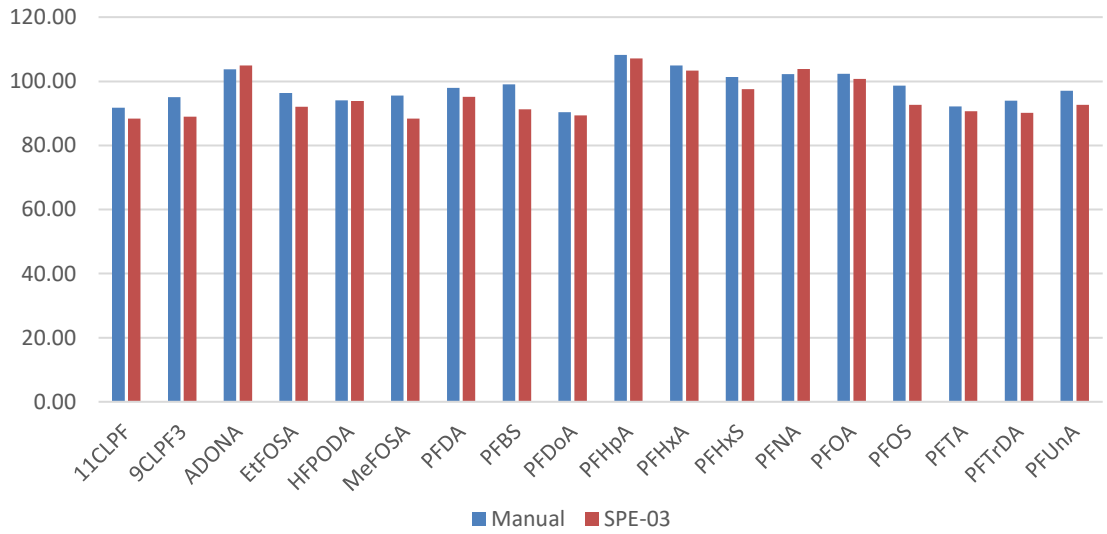

Table 3. Full 18 Analyte Mean Accuracy Comparisons at 50, 20, and 2 ng/L Spiking Levels, n=19

	50 ng/L		20 ng/L		2 ng/L	
Analyte	**Manual**	**Automated**	**Manual**	**Automated**	**Manual**	**Automated**
11CLPF	91.5	92.0	91.3	92.0	91.8	88.3
9CLPF3	95.7	96.2	97.2	96.2	95.1	88.9
ADONA	97.3	105.4	104.1	105.4	103.8	104.9
EtFOSA	93.2	88.6	96.7	88.6	96.4	92.1
HFPODA	90.5	97.0	93.1	97.0	94.1	93.8
MeFOSA	94.0	87.6	94.9	87.6	95.6	88.4
PFDA	94.8	97.8	96.4	97.8	98.0	95.2
PFBS	97.1	92.8	98.5	92.8	99.1	91.2
PFDoA	89.0	91.9	89.8	91.9	90.4	89.4
PFHpA	98.1	104.2	105.5	104.2	108.3	107.1
PFHxA	95.1	101.7	98.2	101.7	104.9	103.3
PFHxS	100.3	102.5	103.1	102.5	101.4	97.6
PFNA	96.1	101.3	100.6	101.3	102.3	103.8
PFOA	97.5	101.4	101.6	101.4	102.4	100.8
PFOS	95.4	94.7	97.0	94.7	98.7	92.7
PFTA	86.7	91.5	86.0	91.5	92.2	90.7
PFTrDA	87.8	92.6	89.2	92.6	94.0	90.2
PFUnA	93.3	95.2	93.6	95.2	97.0	92.7

Table 4. Average Recovery/Precision Comparison of 50, 20, and 2 ng/L LFB, n=19

	Average % Recovery		Average Standard Deviation	
Spike Concentration	**Manual**	**Automated**	**Manual**	**Automated**
50 ng/L	94.2	96.4	7.65	6.15
20 ng/L	96.5	96.3	7.58	7.75
2 ng/L	98.1	95.1	7.75	9.29
Overall Average	96.3	95.9	7.66	7.73

Conclusions

Both the manual and the automated sample preparation techniques, when diligently applied, are shown to be acceptable, analytically-equivalent approaches to the performance of EPA method 537.1. The decision of which technique to apply is primarily an economic choice which balances an individual laboratory's need for high sample throughput (with higher capital cost, but lower per sample labor cost) against lower sample throughput (with lower capital cost, but higher per sample labor cost). Whichever approach is chosen, Strata® SDB-L SPE cartridges, and Luna® C18(2) and Gemini® C18 HPLC columns are the logical choice for EPA method 537.1. Phenomenex SPE cartridges and HPLC columns have proven themselves to be the reliable "work horse" of the PFAS testing industry as demonstrated by the successful analysis of hundreds of thousands of environmental PFAS samples.

Strata® SDB-L (styrene-divinylbenzene) Ordering Information

Format	Sorbent Mass	Part Number	Unit
Tube			
	100 mg	8B-S014-EAK	1 mL (100/box)
	200 mg	8B-S014-FBJ	3 mL (50/box)
	200 mg	8B-S014-FCH	6 mL (30/box)
	500 mg	8B-S014-HBJ	3 mL (50/box)
	500 mg	8B-S014-HCH	6 mL (30/box)
	1 g	8B-S014-JCH	6 mL (30/box)
Giga™ Tube			
	10 g	8B-S014-MFF	60 mL (16/box)
96-Well Plate			
	50 mg	8E-S014-DGB	2 Plates/Box

Gemini® pH Flexible LC Columns Ordering Information

3 µm Microbore, Minibore and MidBore™ Columns (mm)										SecurityGuard™ Cartridges (mm)
Phases	**50 x 1.0**	**20 x 2.0**	**30 x 2.0**	**50 x 2.0**	**100 x 2.0**	**150 x 2.0**	**50 x 3.0**	**100 x 3.0**	**150 x 3.0**	**4 x 2.0*/10pk**
C18	00B-4439-A0	00M-4439-B0	00A-4439-B0	00B-4439-B0	00D-4439-B0	00F-4439-B0	00B-4439-Y0	00D-4439-Y0	00F-4439-Y0	AJ0-7596
C6-Phenyl	—	—	—	00B-4443-B0	00D-4443-B0	00F-4443-B0	00B-4443-Y0	00D-4443-Y0	00F-4443-Y0	AJ0-7914
NX-C18	00B-4453-A0	00M-4453-B0	00A-4453-B0	00B-4453-B0	00D-4453-B0	00F-4453-B0	00B-4453-Y0	00D-4453-Y0	00F-4453-Y0	AJ0-8367

for ID: 2.0-3.0 mm

Luna® Ordering Information

5 µm Microbore and Minibore Columns (mm)						SecurityGuard™ Cartridges (mm)
Phases	**150 x 1.0**	**30 x 2.0**	**50 x 2.0**	**150 x 2.0**	**250 x 2.0**	**4 x 2.0* /10pk**
Silica(2)	—	00A-4274-B0	00B-4274-B0	00F-4274-B0	00G-4274-B0	AJ0-4347
C5	—	00A-4043-B0	00B-4043-B0	00F-4043-B0	—	AJ0-4292
C8(2)	—	00A-4249-B0	00B-4249-B0	00F-4249-B0	00G-4249-B0	AJ0-4289
C18(2)	00F-4252-A0	00A-4252-B0	00B-4252-B0	00F-4252-B0	00G-4252-B0	AJ0-4286
CN	—	—	00B-4255-B0	00F-4255-B0	—	AJ0-4304
Phenyl-Hexyl	—	00A-4257-B0	00B-4257-B0	00F-4257-B0	00G-4257-B0	AJ0-4350
NH_2	—	00A-4378-B0	00B-4378-B0	00F-4378-B0	00G-4378-B0	AJ0-4301
SCX	—	—	00B-4398-B0	—	—	AJ0-4307
PFP(2)	—	00A-4448-B0	00B-4448-B0	00F-4448-B0	—	AJ0-8326

for ID: 2.0-3.0 mm

*SecurityGuard™ Analytical Cartridges require holder, Part No.: KJ0-4282

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