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# Automated On-line SPE-LC/MS/MS Method for Perfluorinated Compounds in Water Samples

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## KEYWORDS

Solid Phase Extraction, LC/MS/MS, Sample Analysis, Lab Automation

## ABSTRACT

Water quality is of the utmost importance and recently the importance of analyzing water for emerging contaminants has been brought to light. Among the emerging compounds being analyzed are perfluorinated chemicals (PFCs) which have been found to be persistent environmental contaminants derived from various industries. For example, perfluorooctane sulfonate (PFOS) has been used in a number of different industries, including the semiconductor and photographic industries, in some firefighting foams and in hydraulic fluids used in the aviation industry. Modern analytical labs are looking to automation to help reduce solvent usage and increase sample throughput while ensuring the high quality of the resulting data.

A single X-Y-Z coordinate autosampler commonly used for sample introduction in GC or HPLC can be used to perform a wide variety of sample preparation techniques using a single instrument and control software. The sampler can also be configured as part of the LC/MS/MS system.

In this report, the complete automation of an on-line SPE-LC/MS/MS method used for the determination of perfluorinated compounds in water samples is discussed. Calibration curves were prepared and limits of quantitation were determined at levels near 0.01 ng/mL for all tested PFCs using a 1 mL sample volume. The average precision for the PFCs examined ranged from 1.73 % to 11.7 % CV and the average accuracy for the PFCs examined ranged from 90.6 % to 110 %.

## INTRODUCTION

Solid phase extraction (SPE) is one of the most widely used methods of sample preparation for chromatographic analysis, as can be seen from the large number of published SPE methods. Typically, a liquid sample is passed across an adsorbent bed to retain and concentrate target analytes while letting sample matrix pass through, eliminating interfering compounds. Alternatively, the adsorbent can be used to retain interfering compounds while allowing target analytes to pass through.

An online SPE procedure was created for the separation, concentration and determination of a select set of perfluorinated compounds from water samples by LC/MS/MS. A GERSTEL MultiPurpose Sampler (MPS) was configured with an online SPE module (SPE$^{XOS}$) and coupled to an Agilent 6470 LC/MS/MS system. The SPE$^{XOS}$ system uses small replaceable cartridges packed with 10-50 mg of sorbent. Concentration of the analytes is achieved by retaining the compounds on the SPE cartridge prior to their elution into the LC/MS/MS system by the mobile phase, resulting in high recovery and high throughput sample cleanup.

## EXPERIMENTAL

*Materials.* Table 1 lists the selected set of perfluorinated compounds used as example compounds and $^{13}$C analogues used as internal standards during this study. All compounds were purchased from Wellington Laboratories.

*Table 1.Perfluorinated compounds (PFCs) examined using on-line SPE-LC/MS/MS Method.*

| Abbrev. | Compound | CAS# | MW [g/mol] | Wellington p/n |
|---|---|---|---|---|
| (PFOA) | Perfluorooctanoic acid | 335-67-1 | 414.0708 | PFOA |
| (PFNA) | Perfluorononanoic acid | 375-95-1 | 464.0786 | PFNA |
| (PFDA) | Perfluorodecanoic acid | 335-76-2 | 514.0864 | PFDA |
| (PFBS) | Perfluorobutanesulfonic acid | 375-73-5 | 300.1 | L-PFBS |
| (PFHxS) | Perfluorohexanesulfonic acid | 355-46-4 | 400.1145 | L-PFHxS |
| (PFOS) | Perfluorooctanesulfonic acid | 1763-23-1 | 500.13 | PFOS |
| Internal Standards | | | | |
| $^{13}$C-PFOA | IntStd_Perfluoro-[1,2-$^{13}$C2]octanoic acid | | 416.0555 (Na) | M2PFOA |
| $^{13}$C-PFOS | IntStd_Sodium perfluoro-[1,2,3,4-$^{13}$C4]octanesulfonate | | 526.0823 (Na) | MPFOS |

Calibration standards were prepared by making the appropriate dilutions of each of the stock solutions listed in Table 1 into LC/MS grade water (Sigma-Aldrich #39253). Each sample was spiked with isotopically labeled internal standards at a concentration of 10 ng/mL. These samples are reported to be stable at room temperature. Following preparation, 10 mL of each spiked water sample was transferred to a 10 mL screw top glass vial for analysis. No septa were included in the vial caps in order to avoid contamination arising from the Teflon coated septa.

SPE$^{XOS}$, C18 HD, 7 µm, SPE cartridges (GERSTEL #018804-001-00) were placed into the SPE$^{XOS}$ online SPE system. The automated method was set up to load a new SPE cartridge for each sample.

*Instrumentation.* Figure 1 shows a graphical representation of the automated online SPE process. The light blue colored flow path shows how the SPE$^{XOS}$ High Pressure Dispenser (HPD), effectively sweeps the sample delivered to the sample loop by the MPS, onto the C18 cartridge mounted in the SPE$^{XOS}$ system. Once the analytes have been concentrated on the cartridge and the cartridge washed, SPE$^{XOS}$ automatically transfers the cartridge from the left side of the unit to the right side in order to bring the cartridge in line with the LC mobile phase. The mobile phase gradient program of the LC/MS/MS system is then used to elute the compounds of interest from the SPE$^{XOS}$, C18 cartridge onto the analytical column were they are separated and subsequently determined. The elution/analysis flow path is represented in dark blue in Figure 1.

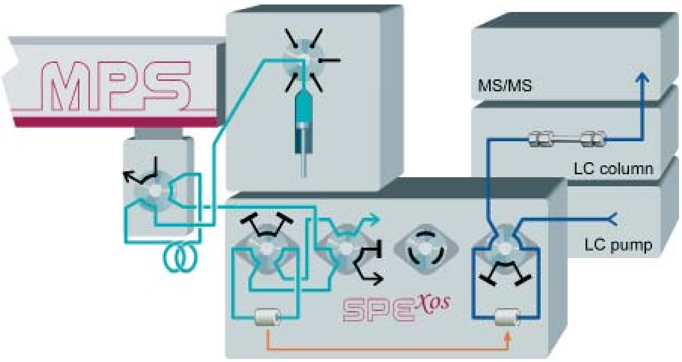

*Figure 2. Graphical representation of the automated online SPE extraction process*

All automated SPE prep sequences were performed using a GERSTEL MPS combined with a GERSTEL SPE$^{XOS}$ system as shown in Figure 2. All analyses were performed using an Agilent 1290 HPLC with an Agilent Eclipse Plus C18 analytical column (2.1 mm x 100 mm, 1.8 µm), an Agilent 6460 triple quadrupole mass spectrometer with Jet Stream electrospray source and GERSTEL MPS autosampler configured with Active Wash Station (AWS). Sample injections were made using a 6 port (0.25 mm) Cheminert C2V injection valve outfitted with a 1.00 mL PEEK sample loop. System components containing Teflon, PTFE, TEF, and other polymers may lead to background contamination. Eliminating, or reducing the number of, such components is key for proper setup of the system as described in EPA Method 537 and Agilent application document 5991-1948EN [1, 2]. A PFC trap column (Agilent XDB C18, 1.8 µm, 4.6 mm x 50 mm) was placed in line between the mobile phase A solvent pump and the jet weaver micro fluidic mixing device, in order to retain any residual contaminants that could interfere with the PFC determination. In addition, tubing of the Active Wash Station and the tubing of the mobile phase bottles were replaced with PEEK tubing with the same inner and outer diameter as the original tubing.

*Figure 2. GERSTEL SPE$^{XOS}$ system.*

*Online solid phase extraction prep sequence*

SPE extraction

1. The SPE$^{XOS}$ system conditions a C18 HD SPE cartridge using 1 mL of acetonitrile followed by 1 mL of water.
2. The MPS injects the water sample into the sample loop of the LC valve.
3. The SPE$^{XOS}$ option loads the sample onto the C18 HD SPE cartridge and washes the cartridge using a total of 6 mL of water.
4. The C18 HD SPE cartridge is brought in-line with the LC mobile phase which is used to elute the compounds of interest, and the LC/MS/MS analysis begins.

*Analysis conditions LC*

| | | |
|---|---|---|
| Mobile phase: | A - 20 mM ammonium acetate in water<br>B - acetonitrile | |
| Gradient: | Initial | 30 % B |
| | 1.0 min | 30 % B |
| | 1.1 min | 50 % B |
| | 6.0 min | 80 % B |
| | 9.0 min | 80 % B |
| | 9.1 min | 30 % B |
| | 10.0 min | 30 % B |
| Flowrate: | 0.2 mL/min | |
| Run time: | 10.0 min | |
| Inj volume: | 1.00 mL | |
| Col. temp.: | 30°C | |

*Analysis conditions MS.*

Electrospray negative mode + Agilent Jet Stream

| | |
|---|---|
| Gas temperature: | 350°C |
| Gas flow (N$_2$): | 5 L/min |
| Nebulizer pressure: | 35 psi |
| Sheath gas temp: | 350°C |
| Sheath gas flow: | 11 L/min |
| Capillary voltage: | -4000 V |
| Nozzle voltage: | -500 V |

The mass spectrometer acquisition parameters and qualifier ions are shown in Table 2.

*Table 2. Mass spectrometer aquisition parameters.*

| Compound | Precursor ion [m/z] | Product ion [m/z] | Dwell [ms] | Fragm. voltage [V] | Coll. energy [V] | Cell acc [V] |
|---|---|---|---|---|---|---|
| APFDA | 513.3 | 469.2 | 30 | 80 | 5 | 5 |
| PFDA | 513.3 | 269.1 | 30 | 80 | 15 | 5 |
| PFDA(3) | 513.3 | 219 | 30 | 80 | 15 | 5 |
| MPFOS | 503.2 | 99 | 30 | 150 | 50 | 5 |
| MPFOS | 503.2 | 80 | 30 | 150 | 50 | 5 |
| PFOS(3) | 499.2 | 168.9 | 30 | 140 | 50 | 5 |
| PFOS | 499.2 | 99 | 30 | 140 | 50 | 5 |
| PFOS | 499.2 | 80 | 30 | 140 | 50 | 5 |
| PFNA | 463.2 | 419.2 | 30 | 80 | 5 | 5 |
| PFNA | 463.2 | 219 | 30 | 80 | 15 | 5 |
| PFNA(3) | 463.2 | 169 | 30 | 80 | 20 | 5 |
| M2PFOA | 415.2 | 370.2 | 30 | 80 | 5 | 5 |
| M2PFOA | 415.2 | 220.1 | 30 | 80 | 15 | 5 |
| M2PFOA(3) | 415.2 | 170 | 30 | 80 | 15 | 5 |
| PFOA | 413.2 | 369.2 | 30 | 80 | 5 | 5 |
| PFOA | 413.2 | 169 | 30 | 80 | 20 | 5 |
| PFHxS(3) | 399.2 | 119 | 30 | 150 | 45 | 5 |
| PFHxS | 399.2 | 99 | 30 | 150 | 45 | 5 |
| PFHxS | 399.2 | 80 | 30 | 150 | 45 | 5 |
| PFBS | 299.1 | 99 | 30 | 130 | 35 | 5 |
| PFBS | 299.1 | 80.1 | 30 | 130 | 35 | 5 |

## RESULTS AND DISCUSSION

The online SPE procedure was shown to successfully prepare samples for subsequent LC/MS/MS analysis and was completely automated using a MAESTRO prep sequence.

In order to achieve the lowest levels of detection, we performed a study to determine the maximum sample volume we could load onto the SPE$^{XOS}$ cartridge. A water sample spiked with 100 ng/mL of each compound was injected using injection volumes of 1.0, 2.0, 3.0, 4.0, and 5.0 mL. Figure 3 shows overlaid mass chromatograms from this experiment. Although only the chromatographic results for PFOS are shown on scale, the remaining peaks behaved similarly. The results of this experiment showed that up to 4 mL sample can be loaded before reaching a saturation point for the SPE$^{XOS}$ cartridge used for solid phase extraction.

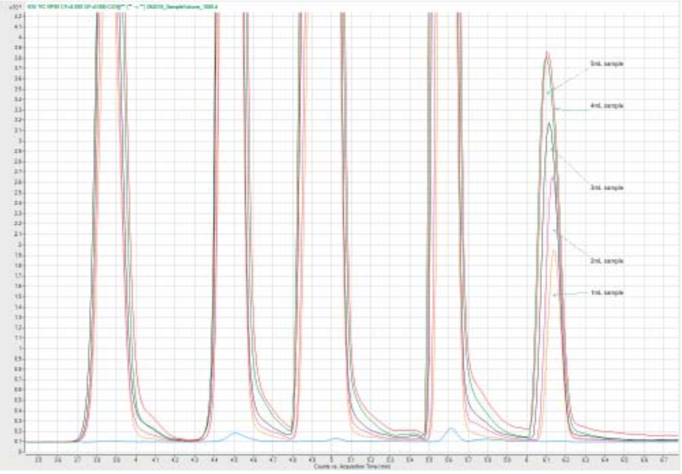

*Figure 3. Overlay chromatograms resulting from increasing volumes of spiked 100 ng/mL water samples.*

Spiked water samples were used for calibration. These ranged in concentration from 0.0100 to 100 ng/mL of the PFC compounds and a volume of 1.0 mL was injected. Table 3 shows the resulting $r^2$ values for each of the PFC compounds monitored as well as the corresponding regression fit used. Figures 4 and 5 show calibration curves for PFOS and PFOA.

*Table 3. Calibration curve results.*

| PFC compound | Regression fit | $r^2$ value |
|---|---|---|
| PFBS | Linear-ignore-1/x | 0.993 |
| PFOA | Quadratic-ignore-1/$x^2$ | 0.988 |
| PFHxS | Linear-ignore-1/$x^2$ | 0.993 |
| PFNA | Linear-ignore-1/$x^2$ | 0.986 |
| PFDA | Quadratic-ignore-1/$x^2$ | 0.993 |
| PFOS | Linear-ignore-1/$x^2$ | 0.995 |

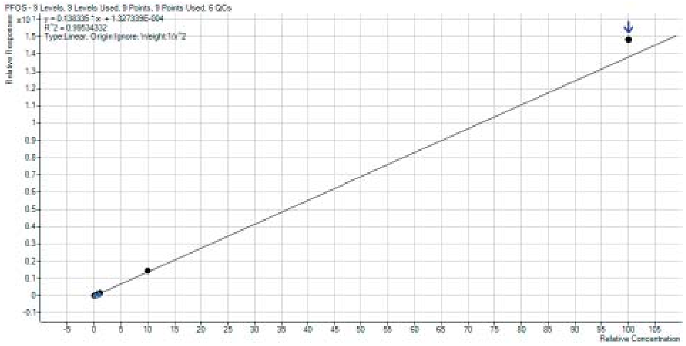

*Figure 4. Representative calibration curve for PFOS.*

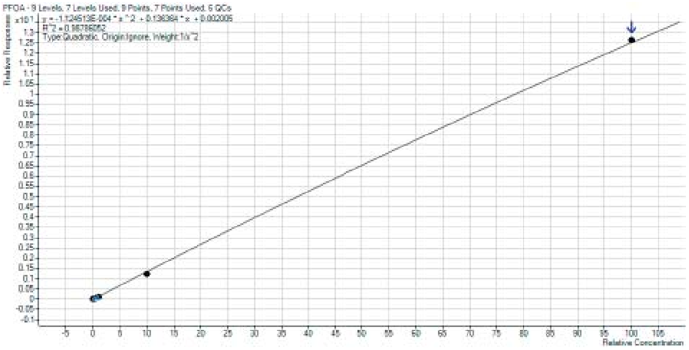

*Figure 5. Representative calibration curve for PFOA.*

The analysis of replicate QC samples was performed to determine precision and accuracy of the complete automated on-line SPE-LC/MS/MS method for perfluorinated compounds in water samples. As shown in Table 4, the average precision for the perfluorinated compounds examined ranged from 1.73 % to 11.7 % CV. The average accuracy for the perfluorinated compounds examined ranged from 90.6 % to 110 %.

*Table 4. Precision and accuracy results.*

| Compound | Level | Exp. conc. [ng/mL] | Final conc. [ng/mL] | Accuracy [%] |
|---|---|---|---|---|
| PFBS | QC 1 | 0.075 | 0.0829 | 110.48 |
| PFBS | QC 1 | 0.075 | 0.0777 | 103.6 |
| PFBS | QC 1 | 0.075 | 0.0765 | 102.03 |
| | | mean | 0.079 | 105 |
| | | SD | 0.00337 | 4.5 |
| | | % CV | 4.27 | 4.27 |
| PFBS | QC 2 | 0.75 | 0.7783 | 103.76 |
| PFBS | QC 2 | 0.75 | 0.8559 | 114.12 |
| PFBS | QC 2 | 0.75 | 0.8317 | 110.89 |
| | | mean | 0.822 | 110 |
| | | SD | 0.03974 | 5.3 |
| | | % CV | 4.83 | 4.838 |
| PFOA | QC 1 | 0.075 | 0.0696 | 92.73 |
| PFOA | QC 1 | 0.075 | 0.0881 | 117.44 |
| PFOA | QC 1 | 0.075 | 0.0799 | 106.51 |
| | | mean | 0.0792 | 106 |
| | | SD | 0.00929 | 12.38 |
| | | % CV | 11.73 | 11.73 |
| PFOA | QC 2 | 0.75 | 0.7211 | 96.15 |
| PFOA | QC 2 | 0.75 | 0.7787 | 103.82 |
| PFOA | QC 2 | 0.75 | 0.6361 | 84.81 |
| | | mean | 0.712 | 94.9 |
| | | SD | 0.0717 | 9.57 |
| | | % CV | 10.1 | 10.1 |
| PFHxS | QC 1 | 0.075 | 0.0838 | 111.66 |
| PFHxS | QC 1 | 0.075 | 0.0749 | 99.87 |
| PFHxS | QC 1 | 0.075 | 0.0806 | 107.5 |
| | | mean | 0.0798 | 106 |
| | | SD | 0.00449 | 5.98 |
| | | % CV | 5.62 | 5.62 |
| PFHxS | QC 2 | 0.75 | 0.7437 | 99.16 |
| PFHxS | QC 2 | 0.75 | 0.7242 | 96.56 |
| PFHxS | QC 2 | 0.75 | 0.7109 | 94.78 |
| | | mean | 0.7263 | 96.8 |
| | | SD | 0.0165 | 2.2 |
| | | % CV | 2.27 | 2.27 |
| PFNA | QC 1 | 0.075 | 0.0737 | 98.26 |
| PFNA | QC 1 | 0.075 | 0.0789 | 105.14 |
| PFNA | QC 1 | 0.075 | 0.0808 | 107.69 |
| | | mean | 0.0778 | 104 |
| | | SD | 0.00366 | 4.88 |
| | | % CV | 4.71 | 4.71 |

*Table 4 (cont.). Precision and accuracy results.*

| Compound | Level | Exp. conc. [ng/mL] | Final conc. [ng/mL] | Accuracy [%] |
|---|---|---|---|---|
| PFNA | QC 2 | 0.75 | 0.6978 | 93.04 |
| PFNA | QC 2 | 0.75 | 0.7135 | 95.13 |
| PFNA | QC 2 | 0.75 | 0.722 | 96.27 |
| mean | | | 0.7111 | 94.8 |
| SD | | | 0.0123 | 1.64 |
| % CV | | | 1.73 | 1.73 |
| PFDA | QC 1 | 0.075 | 0.0702 | 93.57 |
| PFDA | QC 1 | 0.075 | 0.0688 | 91.73 |
| PFDA | QC 1 | 0.075 | 0.0718 | 95.71 |
| mean | | | 0.0703 | 93.7 |
| SD | | | 0.00149 | 1.99 |
| % CV | | | 2.13 | 2.13 |
| PFDA | QC 2 | 0.75 | 0.6308 | 84.1 |
| PFDA | QC 2 | 0.75 | 0.6687 | 89.15 |
| PFDA | QC 2 | 0.75 | 0.7393 | 98.57 |
| mean | | | 0.6796 | 90.6 |
| SD | | | 0.0551 | 7.34 |
| % CV | | | 8.1 | 8.1 |
| PFOS | QC 1 | 0.075 | 0.0636 | 84.78 |
| PFOS | QC 1 | 0.075 | 0.0733 | 97.67 |
| PFOS | QC 1 | 0.075 | 0.0708 | 94.44 |
| mean | | | 0.0692 | 92.3 |
| SD | | | 0.00503 | 6.71 |
| % CV | | | 7.27 | 7.27 |
| PFOS | QC 2 | 0.75 | 0.7185 | 95.8 |
| PFOS | QC 2 | 0.75 | 0.6754 | 90.05 |
| PFOS | QC 2 | 0.75 | 0.6666 | 88.88 |
| mean | | | 0.6868 | 91.6 |
| SD | | | 0.0278 | 3.71 |
| % CV | | | 4.05 | 4.05 |

The signal-to-noise ratio was calculated at the limit of quantitation for each of the perfluorinated compounds examined. Figures 6 and 7 show representative mass chromatograms for PFOS and PFOA at their respective limits of quantitation with the calculated signal-to-noise results. Calculated signal-to-noise and limits of quantitation for all perfluorinated compounds examined are shown in Table 5.

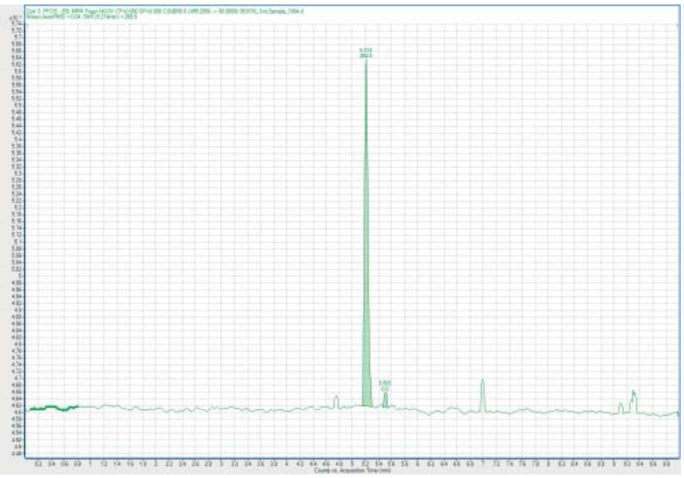

*Figure 6. Signal-to-noise ratio (s/n=269) for PFOS at limit of quantitation (10 pg/mL).*

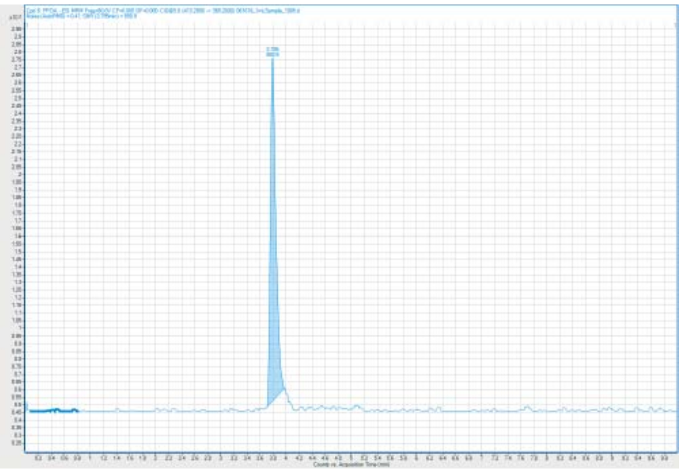

*Figure 7. Signal-to-noise ratio (s/n=551) for PFOA at limit of quantitation (25 pg/mL).*

*Table 5. Limits of quantitation and calculated signal-to-noise ratios.*

| Compound | Limit of quantitation [pg/mL] | Calculated signal-to-noise |
|---|---|---|
| PFBS | 10 | 2480 |
| PFOA | 25 | 551 |
| PFHxS | 10 | 848 |
| PFNA | 10 | 331 |
| PFDA | 25 | 50,039 |
| PFOS | 10 | 269 |

It is important to remember that the background on each analytical system being used during the automated extraction and LC/MS/MS analysis of perfluorinated compounds may be different and may require individual optimization to achieve the desired detection limits. In addition, different perfluorinated compounds may require optimization of both the solid phase extraction procedure and the LC/MS/MS conditions. For the select group of perfluorinated compounds we examined, the conditions were determined to be acceptable.

## CONCLUSIONS

As a result of this study, we were able to show:

- An online SPE procedure successfully prepared samples for subsequent LC/MS/MS analysis and was completely automated using a MAESTRO prep sequence.
- Linear calibration curves with $r^2$ values greater than 0.99 were achieved with limits of quantitation of 10-25 pg/mL.
- The online SPE-LC/MS/MS method proved to be accurate and precise. Average precision for the perfluorinated compounds examined ranged from 1.73 % to 11.7 % CV and average accuracy ranged from 90.6 % to 110 %.

## REFERENCES

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[2] Determination of PFOS and PFOA in Food Matrix of Animal Origin Using UHPLC Hyphenated Triple Quadrupole Tandem Mass Spectrometry, Agilent Technologies, *Application Note 5991-1948EN*, Dunming Xu, et al., Quarantine Technical Centre, Xiamen, China, Meiling Lu, et al., Agilent Technologies, Beijing, China, Xiaogang Chu, China Academy of Inspection and Quarantine, Beijing, China, www.agilent.com/chem.

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