TN-0169

# Use of a Simplified Extraction Method Using a Stacked SPE for Soil Extracts for EPA Method 1633

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## Introduction

PFAS compounds are present in all environmental matrices such as water, soil, air, and living organisms. Due to the persistent nature and toxicity of these compounds, as well as their ability to be easily transported in the environment, there is a significant push to regulate them. The EPA has developed a PFAS Strategic Roadmap to outline the steps needed "to further the science and research, to restrict these dangerous chemicals from getting into the environment, and to immediately move to remediate the problem in communities across the country." One of these steps is to develop and promulgate a standardized method of testing for PFAS in non-drinking water, soil, and biosolid matrices, which is designated as EPA Method 1633.

This method involves a two-step sample preparation approach using a weak anion exchange (WAX) SPE cartridge and graphitized carbon black (GCB) clean-up in a powder format, known as dispersive Solid Phase Extraction (dSPE). In aqueous samples, GCB is added after the SPE step. However, for solid matrices (including, soil, tissues, and other biosolids), the GCB is added before the WAX SPE step. The purpose of the additional GCB clean-up step is to eliminate matrix that can cause interference and reduce bias. GCB has been shown to remove organic acids (such as Humic and Cholic Acids), which can suppress ionization and lead to low bias on the recoveries (especially for PFOS). However, limitations of using GCB are well known in that this media can bind to longer chain PFAS compounds and lead to lower recoveries. This is stated in the EPA Method 1633, "…It is important to minimize the time the sample extract is in contact with the carbon." Besides these practical limitations, adding GCB in a dSPE step is very labor intensive and therefore not practical due to the extra time needed to add, mix, and centrifuge for each sample, especially in high throughput laboratories. In addition, due to the vague guidelines on using GCB for dSPE listed in the 1633 Method, this step can also lead to higher RSD values. However, section 12.2.3 states: "The use of two stacked SPE cartridges, the first containing the WAX sorbent and the second containing activated carbon, or other cartridge configuration may be employed in place of the use of the loose carbon described above."

To sort out this challenge, Strata™ PFAS cartridges were developed as a single cartridge stacked with Strata-X-AW and Strata GCB sorbents that function as a traditional SPE cartridge with a built-in polishing step to meet the method guidelines. We have previously demonstrated the utility of the Strata PFAS stacked SPE format for PFAS analysis following DOD QSM 5.2/Table B15 for a variety of water matrices (TN-0145). We have shown that using a single, stacked WAX/GCB is cheaper, easier, and ultimately yields better recoveries for PFAS analytes from various water samples. This technical note presents a study to demonstrate equivalency of Strata PFAS with GCB followed by SPE WAX for the broader compound list in EPA 1633 and demonstrate the utility for soil extracts.

## Sample Preparation

As a guidance method, EPA Method 1633 makes provisions to demonstrate equivalency as described in section 9.1.2a "… laboratory is permitted certain options to improve separations or lower the costs of measurements. These options include alternative extraction, concentration, and clean-up procedures, and changes in sample volumes, columns, and detectors." Per EPA Method 1633, soil extraction requires using an initial 0.3 % Methanolic Ammonium Hydroxide extraction, GCB clean-up by dSPE, followed by SPE using a WAX cartridge. The method indicates 150 mg of WAX and 10 mg GCB to be used for extraction, but per the method flexibility and previous studies, Strata PFAS was developed with 200 mg of WAX and 25 mg of GCB. Section 11.3 indicates a method for solid sample processing (excluding tissues) and specifically, section 11.3.7 was followed for this study. Section 11.3.7 states: using a 10 mg scoop, add 10 mg of carbon (Section 7.1.17) to the combined extract, mix by occasional hand shaking for 5 minutes and no more, and then centrifuge at 2800 rpm for 10 minutes. Immediately decant the extract into a 60 mL glass evaporation or concentrator tube. This step is not necessary when using the Strata PFAS cartridge.

## LC Conditions

**Column:** Luna™ Omega 3 µm Polar C18
**Dimensions:** 100 x 2.1 mm
**Part No.:** 00D-4760-AN
**Guard Column:** SecurityGuard™ ULTRA for EVO-C18 (AJ0-9296)
**Delay Column:** Luna 5 µm C18(2)
**Delay Column Dimensions:** 30 x 3.0 mm
**Delay Column Part No.:** 00A-4252-Y0
**Mobile Phase:** A: 2 mM Ammonium Acetate in Water / Acetonitrile (95:5, v/v)
B: Acetonitrile

**Gradient:**

| Time (min) | %B |
|---|---|
| 0 | 2 |
| 0.2 | 2 |
| 1 | 25 |
| 7.2 | 30 |
| 9 | 75 |
| 12 | 95 |
| 12.2 | 95 |
| 12.4 | 2 |

**Flow Rate:** 0.4 mL/min
**Injection Volume:** 2 µL
**Temperature:** 40 °C
**LC System:** SCIEX® ExionLC™
**Detection:** MRM
**Detector:** SCIEX QTRAP® 5500+

## MS Conditions

**Ion Source:** TurboV™ Electro Spray Ionization
**Polarity:** Negative
**Source Temperature:** 450 °C
**GS1:** 50 psi
**GS2:** 50 psi
**CUR:** 45 psi
**CAD:** 9 psi
**IS:** -4500 V

## Results and Discussion

To simplify clean-up and SPE into a single step, we used a stacked cartridge that had GCB on top of the WAX SPE. **Figure 1** shows the clean Strata™ PFAS stacked cartridge where the GCB is contained within the cartridge, and the result of using loose GCB on the vacuum manifold. Clean-up time was reduced by not having to remove loose GCB from the vacuum manifold and surrounding surfaces.

Soil samples were spiked with 10 ppt PFAS compounds and then extracted using the method laid out in EPA Method 1633 and the Strata PFAS extraction cartridge. The % recovery results are shown in **Figure 2**. All samples provided excellent recovery and both procedures produced equivalent results. To further validate the equivalency of the Strata PFAS cartridge to the EPA Method 1633 procedure, three Certified Reference Materials (CRM) were extracted and analyzed. First, a QC sample with a known number of PFAS compounds at an unknown concentration was analyzed. As can be seen in **Figure 3**, the % recoveries were equivalent between the two extraction procedures. Next, a proficiency test CRM was analyzed and the % recovery is presented in **Figure 4**. Again, the Strata PFAS extraction cartridge showed comparable % recovery results compared to the standard extraction procedure. Finally, a National Institutes of Standards and Technologies (NIST®) sample was analyzed, and as can be seen in **Figure 5**, the % recoveries were equivalent between the two extraction procedures.

**Figure 1.** SPE Cartridges Used for the Soil Extract Study versus Loose Carbon.

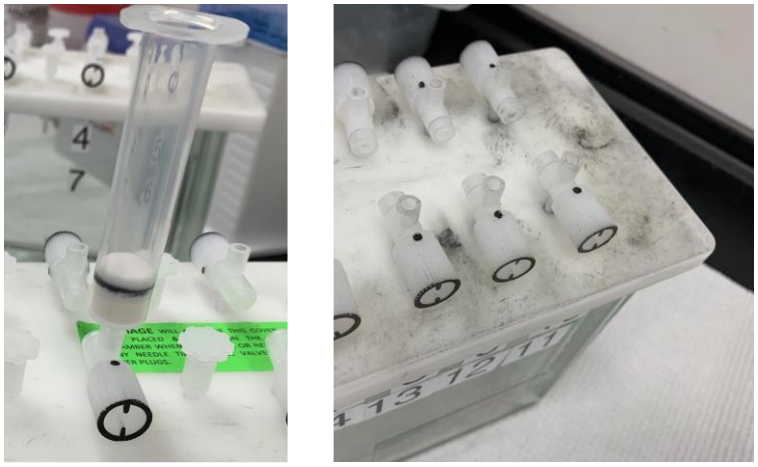


**Figure 2.** PFAS % Recovery Comparison in Spiked Soil Samples.

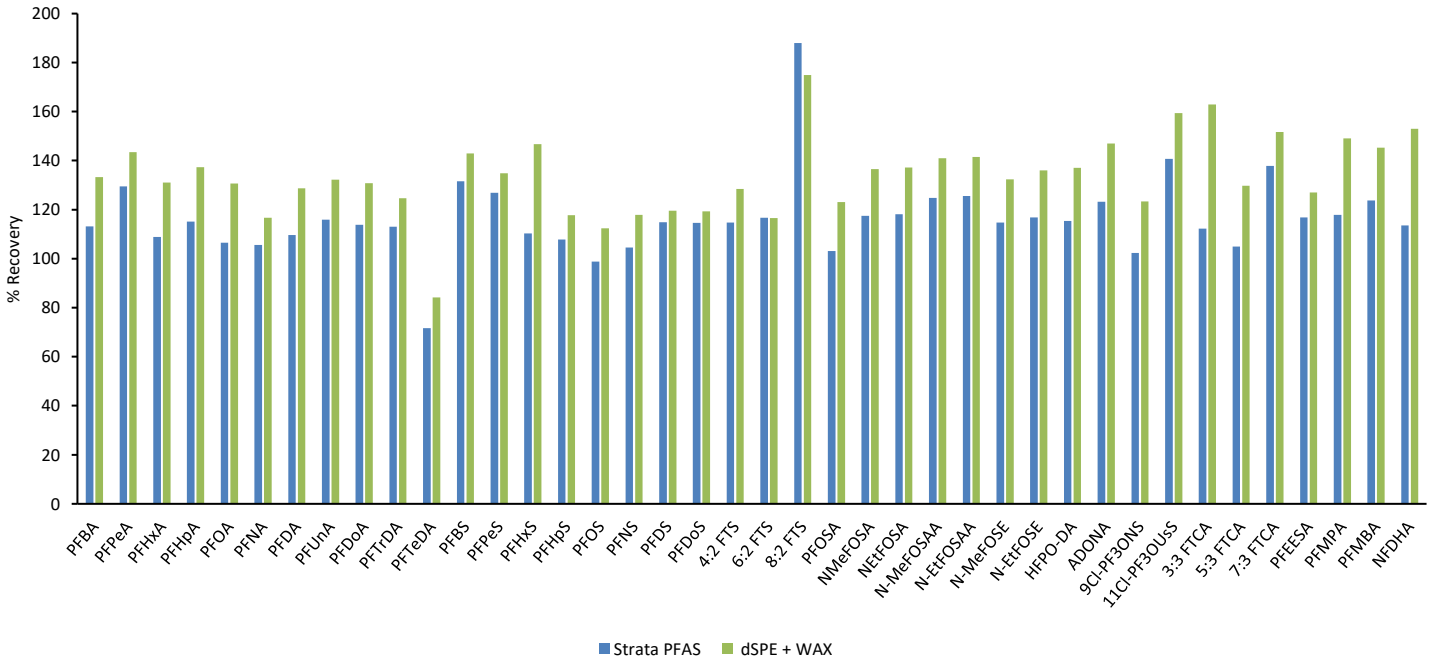


**Figure 3.** PFAS % Recovery Comparison in QC Laboratory Soil Reference Samples.

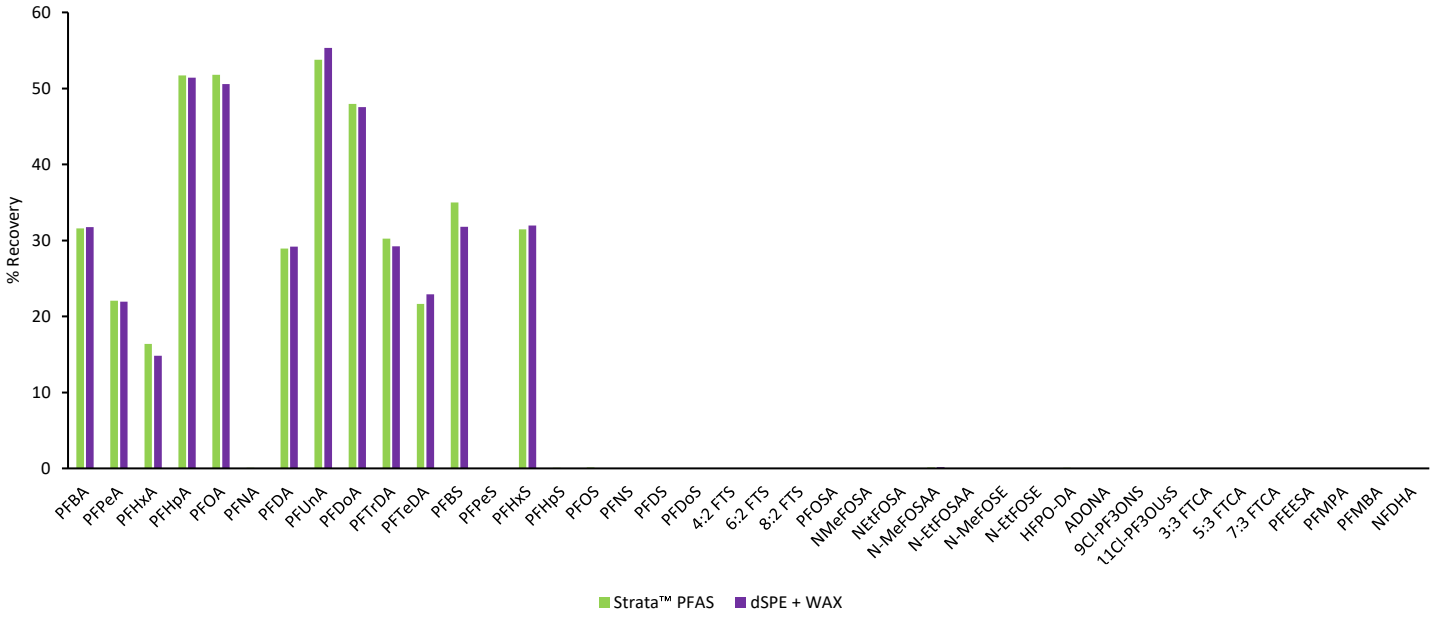



**Figure 4.** PFAS % Recovery Comparison in Proficiency Test Soil Reference Samples.

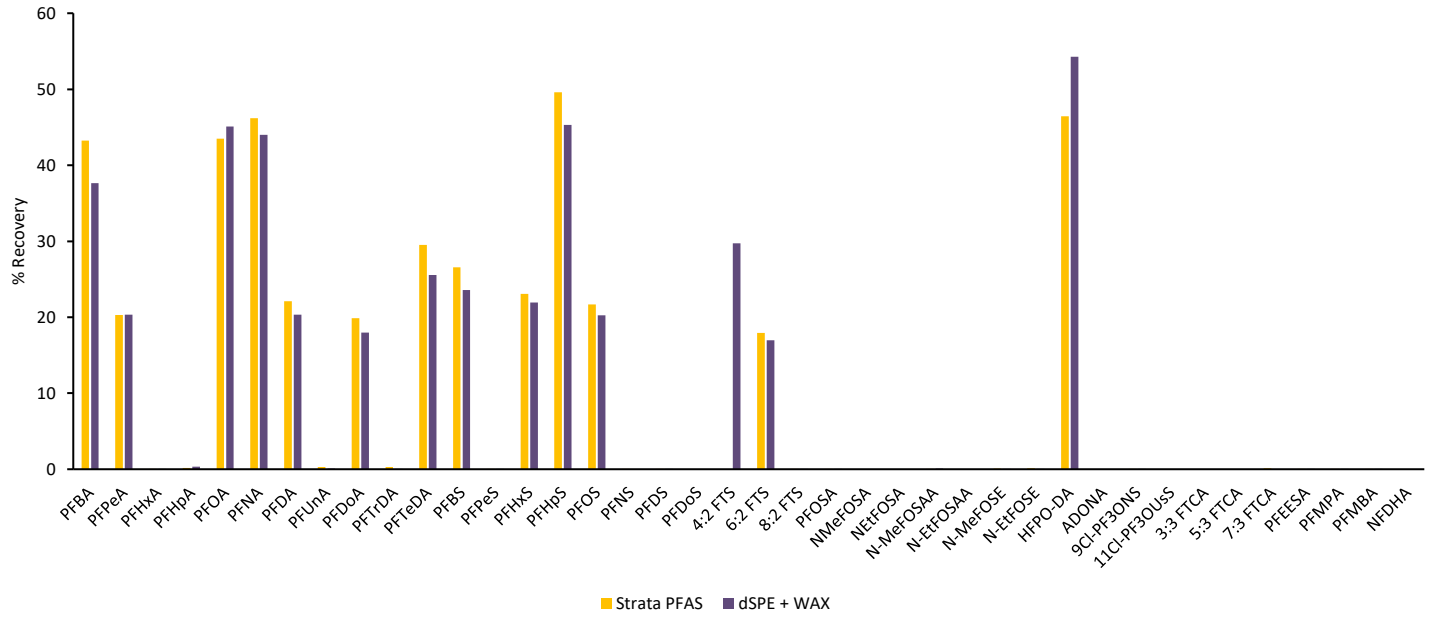
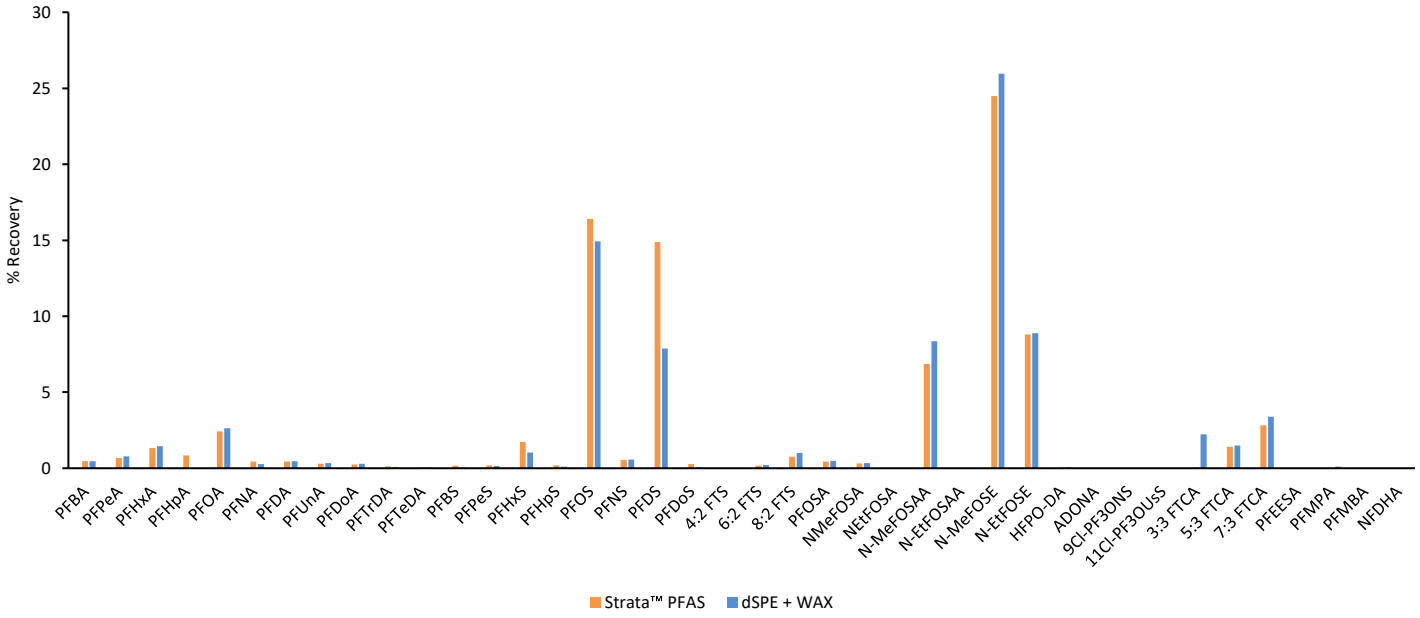


**Figure 5 .** PFAS % Recovery Comparison in NIST® 2781 Soil Reference Samples.




## Conclusions

Two versions of the Strata PFAS stacked cartridges have been developed depending on the sample to be analyzed. For all aqueous matrices, it is recommended to use the WAX/GCB stack order, and for soil, tissues, and biosolids the GCB/WAX stack order. The GCB used in these cartridges mimic where GCB is used with wastewater and soil samples for EPA 1633. Our results demonstrate equivalence of Strata PFAS stacked SPE format (compared to WAX + GCB dSPE) for an EPA Method 1633 PFAS panel from extracts of soil and 3 different soil CRMs. Importantly, Strata PFAS dual layer cartridges (with elimination of dSPE) provides equivalent performance to WAX cartridges specified in EPA Method 1633 for all 40 EPA 1633 parameters. In our laboratory, the elimination of adding GCB in a dSPE step reduces labor per analytical batch (20 samples) by approximately 30 minutes for manual cartridge SPE clean-up. Elimination of the filtration step would provide a further 30 min labor reduction. Incorporation of the dual layer cartridges into the workflow enables automation of the full clean-up procedure, with the potential for a significant reduction in labor and improvements in data reproducibility.

## References

1. Environmental Protection Agency (EPA). Method 1633 Analysis of Per- and Polyfluoroalkyl Substances (PFAS) in Aqueous, Solid, Biosolids, and Tissue Samples by LC-MS/MS.

## Strata™ PFAS Ordering Information

| Strata | | |
|---|---|---|
| **Sorbent Mass** | **Part Number** | **Unit** |
| 200 mg / 50 mg (WAX/GCB) | CS0-9207 | 6 mL (30/box) |
| 500 mg / 50 mg (WAX/GCB) | CS0-9208 | 6 mL (200/box) |
| 50 mg / 200 mg (GCB/WAX) | CS0-9214 | 6 mL (30/box) |
| 250 mg / 50 mg (WAX/GCB) | CS0-9215 | 6 mL (200/box) |
| 250 mg / 100 mg (GCB/WAX) | CS0-9217 | 6 mL (30/box) |
| 200 mg / 10 mg (WAX/GCB) | CS0-9218 | 6 mL (30/box) |

## PFAS CRM Native Standards. All analytes at the same concentration in acid form for easy calculation and dilution.

| Product | Part | Volume | Concentration |
|---|---|---|---|
| EPA 533 mix | AL0-101838 | 1 mL | 2 µg/mL in Methanol |
| EPA 537.1 mix | AL0-101839 | 1mL | 2 µg/mL in Methanol |
| EPA 533 + 537.1 mix | AL0-101840 | 1 mL | 2 µg/mL in Methanol |

Customized CRMs available. Contact Phenomenex for details.

## Other Recommended Products for Your PFAS Methods

| Description | Part No. |
|---|---|
| Luna™ Omega Column 3 µm PS C18 50 x 3 mm | 00B-4758-Y0 |
| Kinetex™ EVO Column 5 µm C18 100 x 2.1 mm | 00D-4633-AN |
| Strata SDB-L 500 mg/6mL tubes, 30/pk | 8B-S014-HCH |
| Verex® Vial, 9 mm Screw, PP, 1.7 mL, 1000/pk | AR0-39P0-13 |
| Verex Vial, 9 mm Screw, PP, 300 µL, 1000/pk | AR0-39P2-13 |
| Verex Vial, 9 mm Screw, PP, 700 µL, 1000/pk | AR0-39P1-13 |
| Vial Cap Verex Cert+ Cap (one piece), 9 mm, PE w/ Starburst pre-Slit, 2mL, 1000/pk | AR0-89P6-13-C |

Columns and vials available in multiple sizes. Contact Phenomenex for details.

## SecurityGuard™ ULTRA Cartridges Ordering Information

| | | | Column ID (mm) | | |
|---|---|---|---|---|---|
| **Material** | **Description** | **pH Stability** | **2.1** | **3.0** | **4.6** |
| | | | /3pk | /3pk | /3pk |
| EVO C18 | (ODS, Octadecyl) | 1.0 – 12.0 | AJ0-9298 | AJ0-9297 | AJ0-9296 |
| C18 | (ODS, Octadecyl) | 1.5 – 8.5* | AJ0-8782 | AJ0-8775 | AJ0-8768 |
| C8 | (MOS, Octyl) | 1.5 – 8.5* | AJ0-8284 | AJ0-8777 | AJ0-8770 |
| PFP | (Pentafluorophenyl) | 1.5 – 8.5* | AJ0-8787 | AJ0-8780 | AJ0-8773 |
| F5 | (Pentafluorophenyl) | 1.5 – 8.5* | AJ0-9322 | AJ0-9321 | AJ0-9320 |
| Biphenyl | (Biphenyl) | 1.5 – 8.5* | AJ0-9209 | AJ0-9208 | AJ0-9207 |
| Phenyl | (Phenylhexyl) | 1.5 – 8.5* | AJ0-8788 | AJ0-8781 | AJ0-8774 |
| HILIC | (HILIC) | 2.0 – 7.5 | AJ0-8786 | AJ0-8779 | AJ0-8772 |
| Polar C18 | (Polar Functional C18) | 1.5 – 8.5* | AJ0-9532 | AJ0-9531 | AJ0-9530 |

*pH stable 1.5 – 8.5 under gradient conditions. pH stable 1.5–10 under isocratic conditions.
AJ0-9000 is the universal holder designed for use with 2.1mm, 3.0mm and 4.6mm ID cartridges.

## Need a different column size or sample preparation format?

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