Application Note

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Benefits of Agilent Altura Ultra Inert HPLC Column Hardware

Analysis of ultrashort-chain and polyfunctional PFAS

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Abstract

Ultrashort-chain (USC) per- and polyfluoroalkyl substances (PFAS) present significant analytical challenges due to their high polarity, acidity, and poor retention on conventional reversed-phase columns. The Agilent Altura Poroshell 120 PFAS (Altura Poroshell PFAS) column was developed to address these challenges through optimized stationary phase design, enabling improved retention of these compounds in aqueous matrices. In addition, the Altura columns incorporate Ultra Inert technology, which provides an inert flow path that minimizes interactions between analytes and metal surfaces. This could be important for highly acidic compounds, which can otherwise adsorb onto stainless steel and negatively impact chromatographic performance.

This study evaluates the impact of Ultra Inert technology on the analysis of USC PFAS by comparing a stainless steel column packed with the Poroshell PFAS phase to an equivalent configuration using the Altura Ultra Inert hardware. The effects of injection volume and matrix composition were also investigated. Nine conventional USC PFAS were analyzed alongside two polyfunctional difluorinated analytes—difluoromalonic acid (MMF) and difluorosulfoacetic acid (DFSA)—to further assess the effect of hardware.

The Altura hardware improved peak area and retention time reproducibility compared to stainless steel. Seven of the nine USC PFAS compounds exhibited increased peak areas with Altura hardware. While stainless steel columns delivered acceptable performance, the Altura configuration demonstrated greater consistency across injection volumes and water matrices. Enhanced reproducibility was evident for MMF and DFSA, where the Altura configuration helped maintain more consistent retention behavior and more interpretable peak shape. These results highlight the Altura hardware can improve robustness and confidence in USC PFAS workflows.

Introduction

USC PFAS are being increasingly detected in environmental samples.[1] As small, highly polar, acidic compounds, they present a significant chromatographic challenge due to their limited retention on conventional C18 columns. Agilent has addressed this issue with the Agilent Altura Poroshell 120 PFAS column, which has demonstrated excellent retention of these compounds in environmental water samples.[2]

An additional benefit of this column is its Ultra Inert technology. Inert column hardware has shown substantial advantages for the analysis of biomolecules—including organic acids, phosphorylated nucleotides, and phosphopeptides—that can interact with stainless steel surfaces. These interactions may lead to analyte adsorption (resulting in reduced signal) and pronounced peak tailing.[3] This mechanism is relevant to USC PFAS because many of these analytes are strongly acidic and are measured at low concentrations in matrices that can further influence adsorption or ionization.

USC PFAS include strong acids such as trifluoroacetic acid (TFA) and may therefore benefit from inert column hardware. This application note investigates the effect of inert column hardware on USC PFAS by comparing a stainless steel column packed with the Poroshell PFAS phase to an equivalent column configured with the Altura Ultra Inert hardware.

Injection volume and matrix type were also evaluated to determine whether these parameters interact with column hardware. Nine conventional USC PFAS were included in the study, along with two additional compounds— difluoromalonic acid (MMF) and difluorosulfoacetic acid (DFSA)—commonly used in fluorinated chemistry synthesis. These additional analytes were incorporated to provide a more challenging test case for both the chromatographic method and the column hardware. Both compounds are very small, highly polar, polyfunctional compounds that can be especially sensitive to method parameters.

Methods

Chemicals and solvents

Table 1 summarizes the compounds included in the study along with their respective suppliers. Acetic acid and ammonium acetate were obtained from Sigma-Aldrich. The Agilent InfinityLab Acetonitrile for LC/MS (p/n 5191-5101) and InfinityLab Methanol for LC/MS (p/n 5191-5111) were used as mobile phase solvents. The Agilent InfinityLab Water for LC/MS (p/n 5191-5121) was used for mobile phase preparation. All other chemicals were sourced from VWR.

Table 1. Target compounds and suppliers.

Compound name	Abbreviation	Compound Formula	CAS Number	Supplier
$^{13}C_2$-TFA	13C2-TFA	$[^{13}C]_2HF_3O_2$	NA	Cambridge Isotopes
$^{13}C_3$-PFPrA	13C3-PFPrA	$[^{13}C]_3HF_5O_2$	NA	Wellington Labs
Pentafluoroethanesulfonic Acid	PFEtS	$C_2F_5SO_3H$	354-88-1	Wellington Labs
Trifluoromethanesulfonic Acid	PFMeS	CF_3SO_3H	1493-13-6	Wellington Labs
Perfluoro-2-methoxyacetic Acid	PFOMAA	$C_3F_5O_3H$	674-13-5	Cambridge Isotopes
Pentafluoropropionic Acid	PFPrA	$C_3F_5O_2H$	422-64-0	Cambridge Isotopes
Perfluoropropanesulfonic Acid	PFPrS	$C_3F_7SO_3H$	423-41-6	Wellington Labs
Trifluoroacetic Acid	TFA	$C_2F_3O_2H$	76-05-1	Cambridge Isotopes
Difluoroacetic Acid	DFA	$C_2H_2F_2O_2$	381-73-7	Sigma-Aldrich
Heptafluorobutyric Acid	PFBA	$C_4HF_7O_2$	375-22-4	Wellington Labs
Trifluoromethanesulfonyl (TFSI)	TFSI	$C_2F_6NO_4S_2$	90076-65-6	Accustandard
Additional Compounds				
$^{13}C_3$-MMF	13C3-MMF	$[^{13}C]_3F_2O_4H_2$	NA	Cambridge Isotopes
Difluoromalonic Acid	MMF	$C_3H_2F_2O_4$	1514-85-8	Cambridge Isotopes
Difluoro(sulfo)acetic Acid	DFSA	$C_2H_2F_2O_5S$	422-67-3	Santa Cruz Biotechnology

Test samples and study design

Water test samples consisted of reverse osmosis (RO) water and synthetic water (SW). The SW matrix was prepared based on a formulation suggested in EPA Method 5574 to evaluate performance in samples with high anion concentrations. SW was prepared using sodium salts to achieve the following concentrations: 20 mg/L nitrate, 150 mg/L bicarbonate, 250 mg/L chloride, and 250 mg/L sulfate. In addition to the two water types, test samples were prepared in LC/MS-grade methanol (MeOH).

The study design is shown in Figure 1. Three concentrations of USC PFAS were prepared in each sample matrix, with levels adjusted based on injection volume. Three replicate injections of each concentration were performed under each set of study conditions.

Analytical columns included a commercially available Altura Poroshell PFAS column and a custom-packed stainless steel column with the same Poroshell PFAS stationary phase (SS). Because the solid phase material originated from the same batch, the comparison was designed to isolate the effect of column hardware inertness.

Instrumentation

Analysis was performed using an Agilent 1290 Infinity II LC system coupled to an Agilent 6475 triple quadrupole LC/MS. To reduce PFAS contamination and background originating from solvents and the LC system, an Agilent InfinityLab PFAS analysis HPLC conversion kit (p/n 5004-0006) was installed. However, the Agilent InfinityLab PFC delay column (p/n 5062-8100) supplied with the kit was replaced with an Agilent Poroshell 120 PFAS delay column (p/n 027403-007).

While the standard PFC delay column utilizes a conventional C18 phase, analysis of USC PFAS requires a mixed-mode stationary phase, such as that in the Poroshell PFAS delay column, to effectively retain and delay compounds such as TFA.

Table 2 summarizes the LC conditions and Tables 3 and 4 present the MS source parameters and MRM transitions, respectively.

Table 2. LC conditions.

Parameter	Value
Test Columns	– Agilent Altura PFAS 2.1 × 50 mm, 2.7 µm (p/n 227210-007) – PFAS Media packed into 2.1 × 50 mm stainless steel hardware
Delay Column	Poroshell 120 PFAS Delay Column, 4.6 × 30 mm (p/n 027403-007)
Column Temperature	40 °C
Injection Volume	5/20 µL
Autosampler Temperature	20 °C
Standard Wash	1:1 Methanol:water, 5 sec flush
Mobile Phase	A) 0.1% Acetic acid in water B) 1:1 Acetonitrile:MeOH + 5% water+ 20 mM ammonium acetate
Flow Rate	0.5 mL/min
Gradient Program	Time (min) %B 0 10 0.5 10 2 40 6 40 10 100 12.1 100 13 10 14 100 14.1 10
Stop Time	14.50 min
Post Time	4.00 min

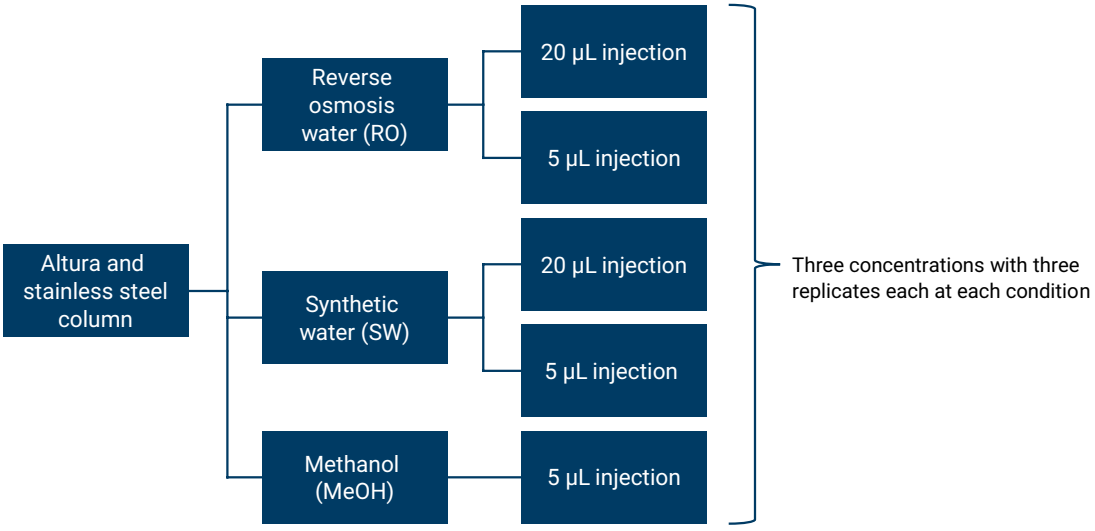

Figure 1. Study design.

Table 3. 6475 LC/TQ source conditions.

Parameter	Value
Parameter	Setting
Gas Temperature	200 °C
Drying Gas Flow	9 L/min
Sheath Gas Temperature	400 °C
Sheath Gas Flow	11 L/min
Nebulizer	30 psig
Capillary Voltage	2,000 V
Nozzle	0 V

Statistical analysis

Statistical analysis was performed using RStudio (version 2026.01.2, Build 418). Compound peak areas were normalized for concentration, followed by $\log_{10}$ transformation. Injection volume, column type, and sample matrix were treated as categorical predictors, and interaction effects were included in the fitted linear regression models. Analysis of variance (ANOVA) tests were performed on the models to evaluate the significance of each predictor.

Table 4. 6475 LC/TQ MRM transitions.

Compound Name	Precursor *m/z*	Product *m/z*	RT (min)	RT Window (min)	Fragmentor (V)	CE (V)	Polarity
DFA	95	51	2.58	2	70	10	Negative
13C2-TFA	115	69.9	3.08	2	70	10	Negative
TFA	113	68.9	3.08	2	70	10	Negative
TFA	113	19	3.08	2	70	22	Negative
PFMeS	149	98.9	3.43	2	115	30	Negative
PFMeS	149	79.9	3.43	2	115	26	Negative
PFPrA	163	119	3.93	2	70	10	Negative
PFPrA	163	68.9	3.93	2	70	40	Negative
PFPrA	163	19	3.93	2	70	14	Negative
13C3-PFPrA	166	121	3.94	2	70	10	Negative
PFMOAA	179	84.9	4.3	2	60	10	Negative
PFEtS	199	98.9	4.44	2	115	30	Negative
PFEtS	199	79.9	4.44	2	115	34	Negative
PFBA	213	169	5.27	2	72	8	Negative
PFBA	213	19	5.27	2	72	13	Negative
13C3-PFBA	216	172	5.28	2	72	8	Negative
PFPrS	249	98.9	6.24	2	135	30	Negative
PFPrS	249	98.9	6.24	2	135	30	Negative
PFPrS	249	79.9	6.24	2	135	36	Negative
TFSI	279.9	210.9	8.25	2	115	20	Negative
TFSI	279.9	146.9	8.25	2	115	30	Negative
TFSI	279.9	77.9	8.25	2	115	40	Negative
DFSA	175	130.9	8.6	4	80	10	Negative
DFSA	175	80.9	8.6	4	80	20	Negative
13C3-MMF	142	96.9	9.2	4	80	6	Negative
13C3-MMF	142	52	9.2	4	80	14	Negative
MMF	139	94.9	9.2	4	70	6	Negative
MMF	139	50.9	9.2	4	70	14	Negative

Results and discussion

Chromatography and retention time performance

Chromatographic conditions were initially based on previously published work.[2] However, the inclusion of MMF and DFSA required further optimization to achieve acceptable performance. The addition of methanol improved ionization efficiency, particularly for DFSA, while increased concentrations of ammonium acetate and acetonitrile were used to balance gradient length and chromatographic performance.

Due to significant laboratory background levels of TFA, the isotopically labeled analogue, $^{13}C_2$-TFA, was used as a surrogate for TFA in all comparisons. Figure 2 shows chromatograms of the same concentration in RO water acquired using Altura and stainless steel column hardware. All peak shapes appear Gaussian for both columns. A slight forward-retention-time shift is visible for DFA and $^{13}C_2$-TFA, whereas substantially larger shifts are evident for MMF and DFSA, highlighting their sensitivity to method parameters.

Results indicate that MMF and DFSA are highly sensitive to interaction effects among column hardware, injection volume, and sample matrix. These effects are discussed separately in a dedicated section.

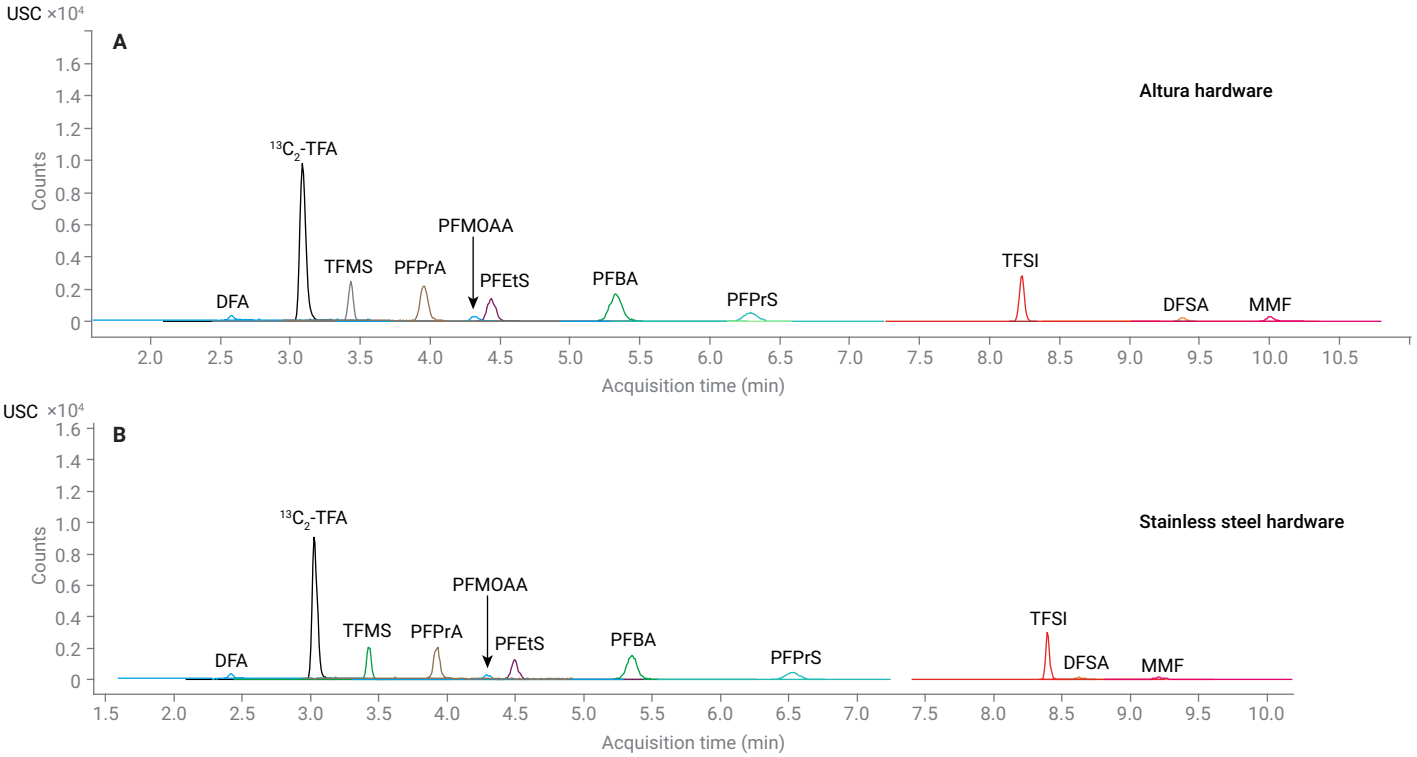

Figure 2. Compound chromatograms in RO water. (A) Altura hardware (B) Stainless steel hardware.

When considering retention time (RT) stability based solely on the column, the Altura hardware demonstrated more consistent performance. Figure 3 presents the RT relative standard deviation (RSD), averaged across injection volume and sample matrix, for each column hardware type. Although both the Altura and stainless steel hardware provided acceptable performance, the Altura hardware was less affected by variations in method parameters, resulting in a more robust and reliable method.

Statistical analysis modeling effect of column hardware on peak area

Peak area was used as the primary metric to assess compound performance as no tailing was observed with stainless steel. Following data normalization, statistical analysis was performed to isolate the effect of column hardware while accounting for other method factors. Interaction plots were generated to visually evaluate trends in normalized data.

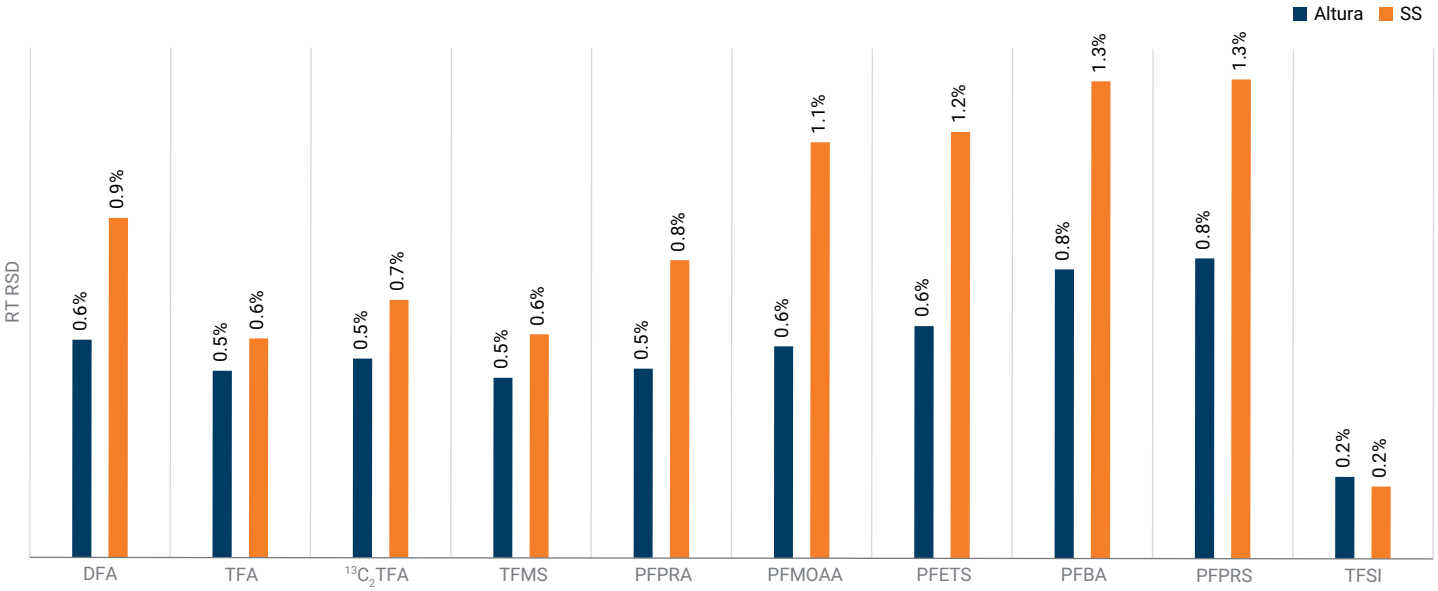

Figure 3. Comparison of RT RSD across all matrices for the Agilent Altura and stainless steel hardware.

Figure 4 presents representative interaction plots for PFPrA and PFBA, illustrating the contrast between the two compounds. For PFPrA (A; $p < 0.001$), the column hardware term explains a significant portion of the variance in compound abundance, indicating that column hardware impacts abundance after correction for other factors, whereas for PFBA (B) it does not. These plots further stratify column effects by injection volume and sample matrix.

Notably, the PFPrA plot shows that the difference between column hardware becomes more pronounced at the 20 µL injection volume for both SW and RO matrices. This pattern reflects a significant interaction between injection volume and column type ($p < 0.001$), indicating that the impact of column hardware on compound abundance depends on injection volume and is greater at higher injection volumes for this compound.

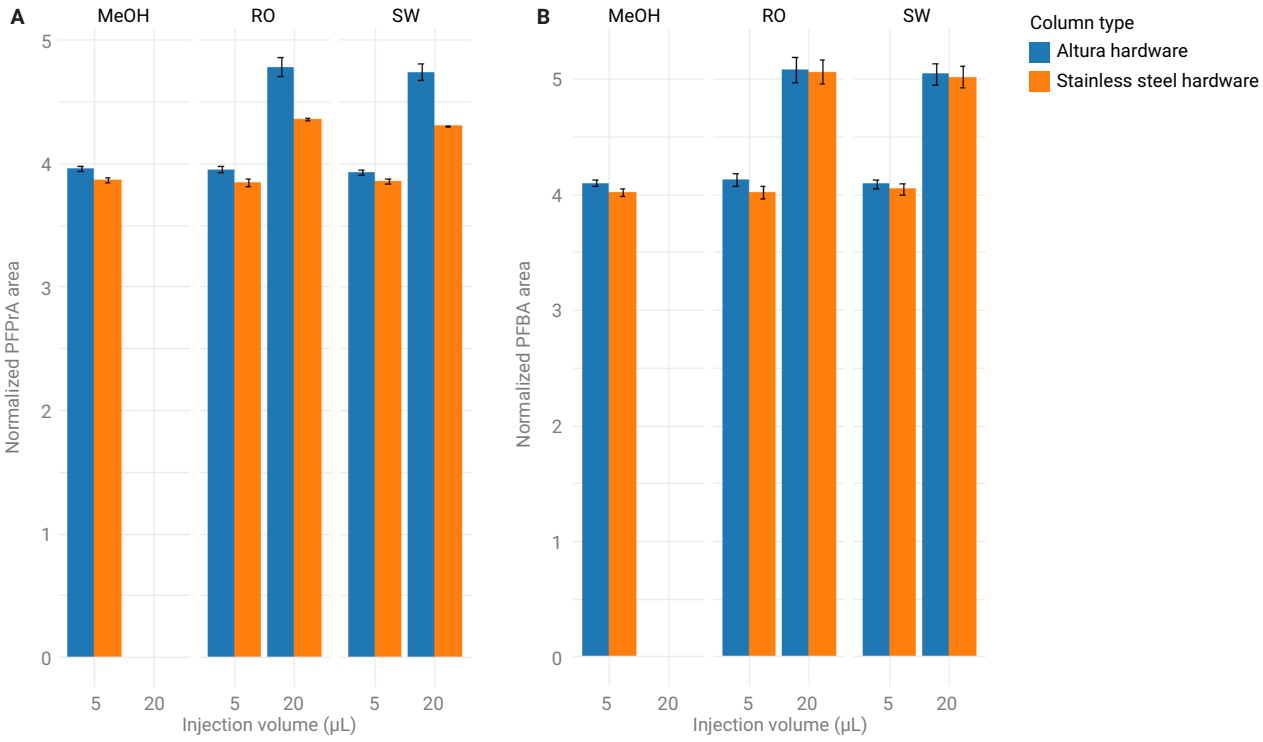

Figure 4. Interaction plots of column hardware, injection volume, and matrix, with bars representing standard error. (A) PFPrA shows a significant impact of column hardware and (B) PFBA does not show a significant impact of column hardware.

Closer examination of the PFPrA data reveals an additional benefit of the Altura hardware: reduced transition background for PFPrA. Figure 5 demonstrates that Altura hardware can lower MS background levels and improve the signal-to-noise (S/N) ratio for the compound.

The statistical significance and magnitude of the factor improvement attributable to column hardware, after correcting for other variables, are shown in Figure 6. Overall, column hardware had a statistically significant effect on seven of the nine standard USC PFAS compounds evaluated. The largest improvements were observed for $^{13}C_2$-TFA and PFMOAA. Factor increases range from 1.007 to 1.23x, supporting the hypothesis that inert column hardware can improve abundance for USC PFAS.

PFBA and DFA did not exhibit a statistically significant dependence on column hardware. For DFA, the response was primarily influenced by sample matrix, with evident signal suppression in SW samples, consistent with previous observations.[2]

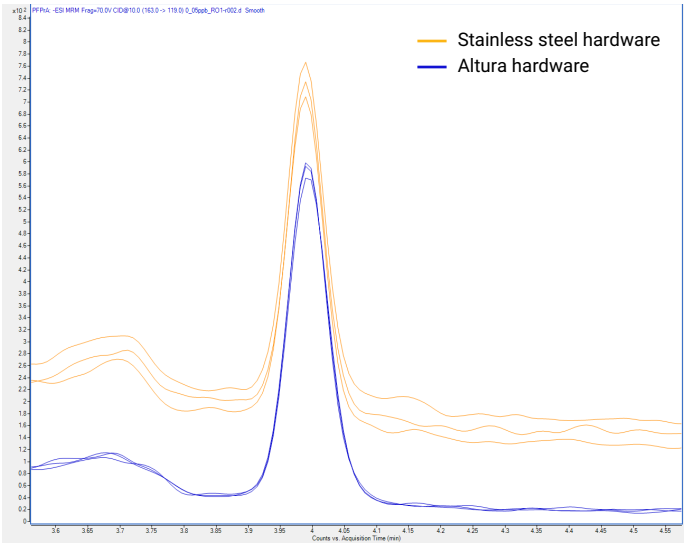

Figure 5. PFPrA chromatogram, 20 µL injection, 50 ng/L in RO water. The observed S/N ratio for stainless steel hardware was 17 and for Altura hardware, it was 22.

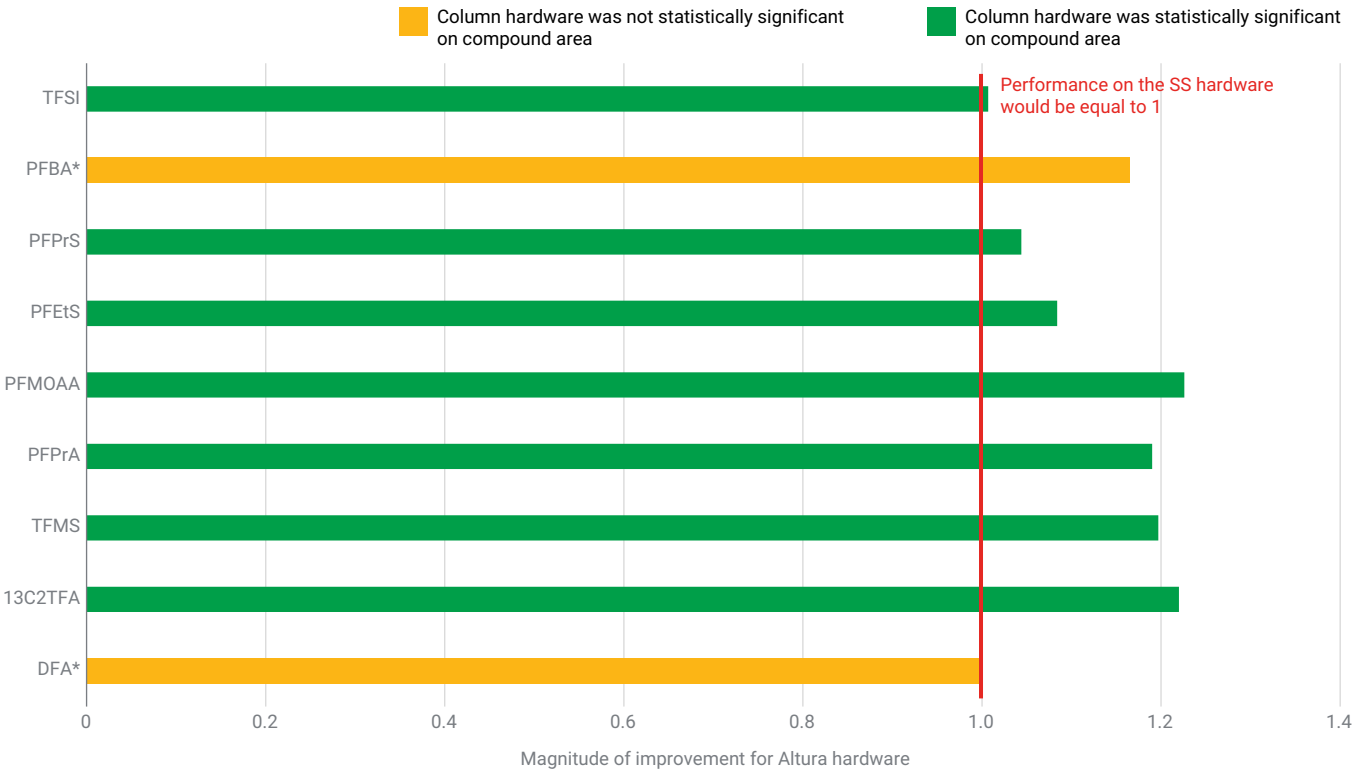

Figure 6. Factor abundance increase provided by the Agilent Altura column hardware in comparison to stainless steel (*indicates the effect was not statistically significant based on the model).

DFSA and MMF

DFSA and MMF were highly sensitive to interaction effects among injection volume, column type, and matrix, particularly at higher injection volumes. At an injection volume of 5 µL (Figures 7A and 7B), a clear forward shift in RT is observed between the two column hardware types and across different matrices. The shift between RO and SW matrices is more pronounced with stainless steel hardware. However, all peaks remain present and distinguishable.

At a larger injection volume of 20 µL (Figures 7C and 7D), peak behavior in SW becomes problematic, particularly for DFSA. Although a peak distinguishable from the blank is observed, it is significantly shifted forward and exhibits enhanced response and notable tailing. Due to the unavailability of a labeled analogue of DFSA, it remains unclear whether this peak definitively corresponds to DFSA, given the observed shift and signal enhancement.

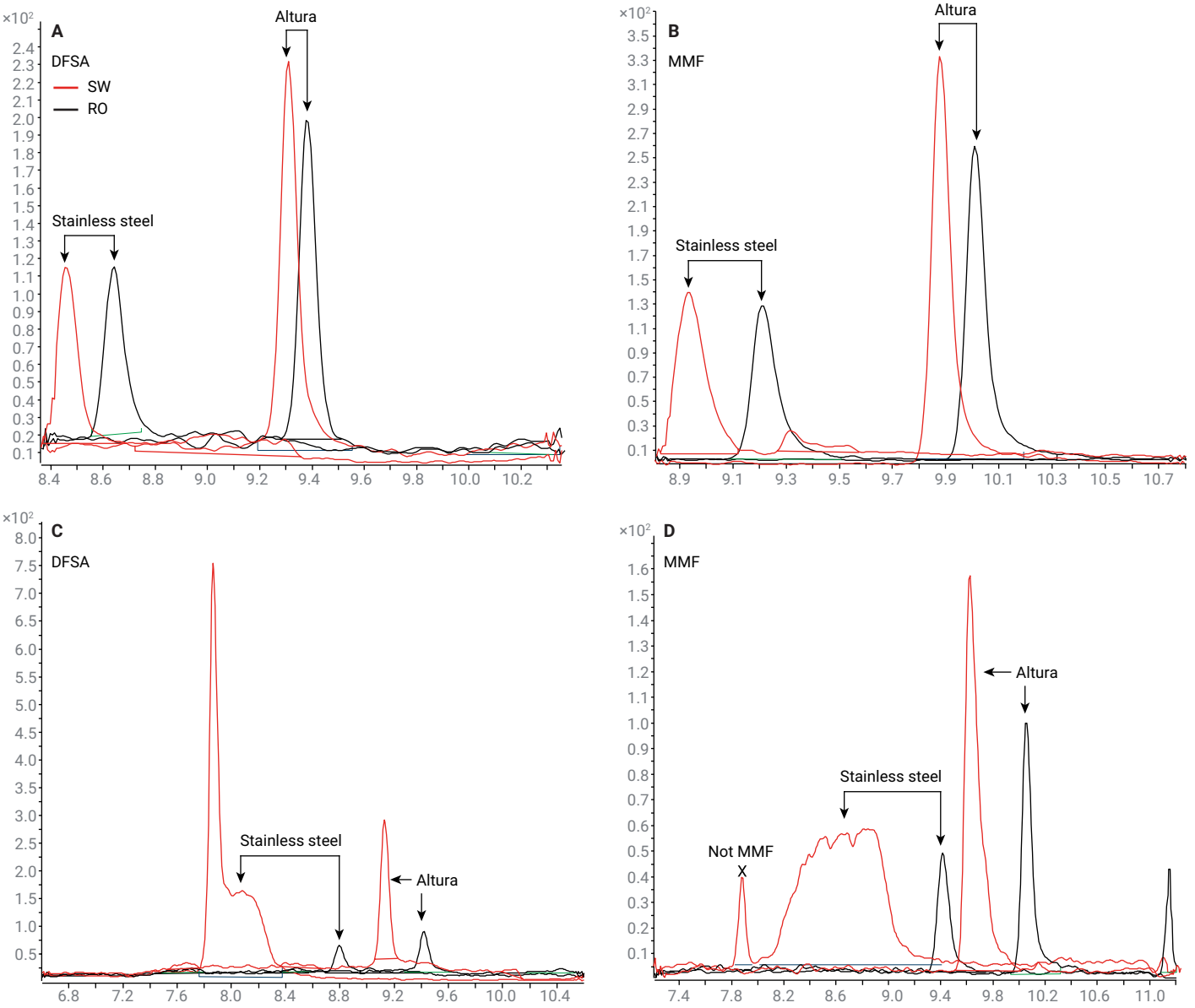

Figure 7. Chromatogram overlay of different method conditions for MMF and DFSA: (A) DFSA at 5 µL injection (B) MMF at 5 µL injection (C) DFSA at 20 µL injection (D) MMF at 20 µL injection.

Even though a labeled analogue was available for MMF, under SW conditions with stainless steel hardware, the peak appears to degrade into a smear rather than a Gaussian distribution. In contrast, Altura hardware maintained a more defined peak profile, supporting its advantage in preserving usable chromatography for highly polar, matrix-sensitive analytes.

Overall, these results indicate that both DFSA and MMF are not well suited to large-volume injections. Performance is more consistent and reproducible when using inert column hardware, such as Altura.

Conclusion

The Altura hardware demonstrated improved peak area and RT reproducibility compared to stainless steel. Seven of the nine USC PFAS standard compounds showed increased peak area with the Altura hardware. While performance using stainless steel hardware was acceptable, the inert hardware provided greater consistency across different water matrices and injection volumes. Additionally, when incorporating more sensitive analytes such as DFSA and MMF, the Altura coating again delivered more consistent performance across varying method conditions and sample types.

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