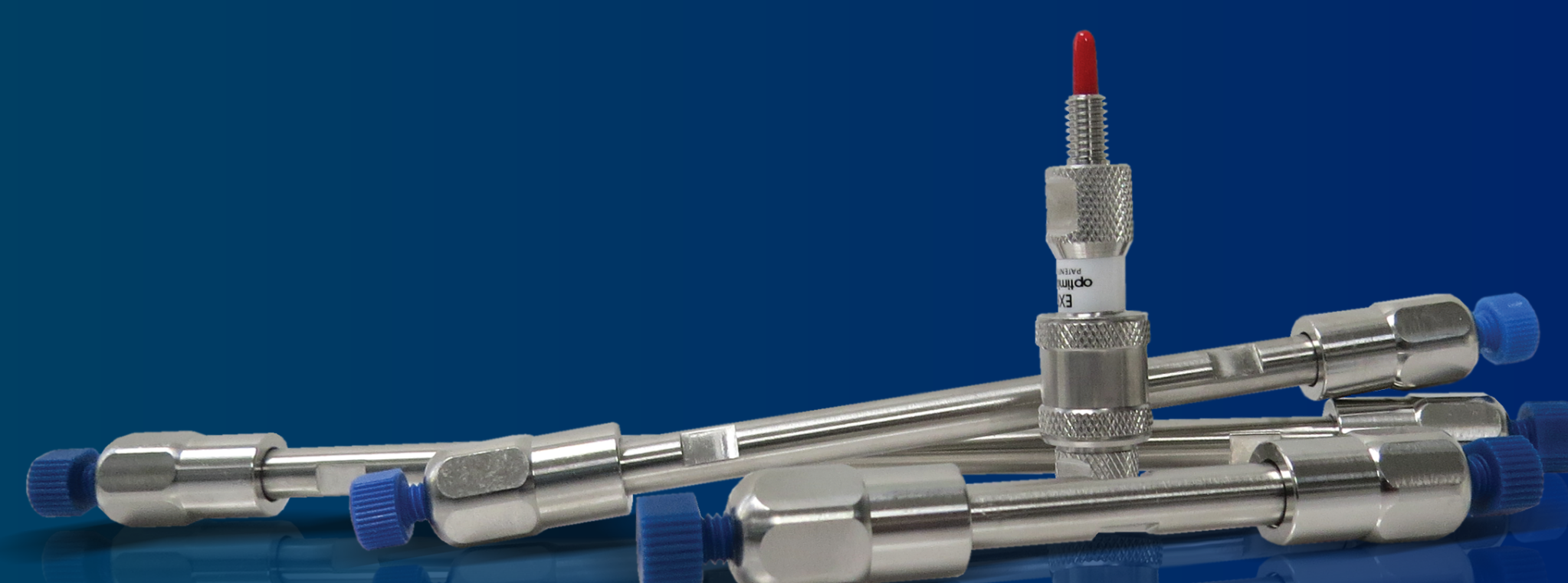




# EPA Method 1633: Analysis of PFAS & Separation of Bile Salts Using SelectraCore® C18 HPLC Column by LC-MS/MS

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## SUMMARY

PFAS, a group of synthetic organofluorine compounds widely used in industry and consumer products, are persistent and bioaccumulative. EPA 1633, introduced in 2021, expanded EPA's methods to measure 40 PFAS analytes across different matrices [1]. The method, updated in January 2024, mandates a 1-minute separation between TDCA and PFOS [2]. This study investigates PFAS analysis per EPA 1633 using UCT's SelectraCore® C18 HPLC column, overcoming challenges to optimize separation without compromising runtime or other compound integrity achieving a 0.5-minute shorter runtime than EPA's method while maintaining a 1.4-minute TDCA-PFOS separation.

**References**  
[1] Draft Method 1633 Analysis of Per- and Polyfluoroalkyl Substances (PFAS) in Aqueous, Solid, Biosolids, and Tissue Samples by LC-MS/MS (USEPA, January 2024)  
[2] Report on the Multi-Laboratory Validation of PFAS by Isotope Dilution LC-MS/MS, Wastewater, Surface Water, and Groundwater (Strategic Environmental Research and Development Program (SERDP) Project ER19-1409. (Wiley, J., T. Thompson, A. Hanley, R. Anderson, T. Thompson, and A. Leeson, 2023))

## HPLC CONDITIONS

|                  |                                                                                                          |
|------------------|----------------------------------------------------------------------------------------------------------|
| HPLC System      | SCIEX Exion LC                                                                                           |
| Delay Column     | UCT Selectra® C18, 50 × 4.6 mm, 5 µm (p/n: SLC-1850ID46-5UM)                                             |
| HPLC Column      | UCT SelectraCore® C18, 100 × 2.1 mm, 2.7 µm (p/n: SCS27-C181021)                                         |
| Guard Column     | UCT SelectraCore® C18, 5 × 2.1 mm, 2.7 µm (p/n: SCS27-C18GDC21) Guard column holder p/n: SLGRDHLDR-HPOPT |
| Column Temp.     | 40°C                                                                                                     |
| Injection Volume | 3 µL                                                                                                     |

### GRADIENT PROGRAM

| Time (min) | Mobile Phase A (%): 95% 2mM Ammonium Acetate (aq)/5% Acetonitrile | Mobile Phase B (%): Acetonitrile | Flow Rate (µL/min) |
|------------|-------------------------------------------------------------------|----------------------------------|--------------------|
| 0.0        | 98                                                                | 2                                | 0.35               |
| 0.2        | 98                                                                | 2                                | 0.35               |
| 4          | 70                                                                | 30                               | 0.4                |
| 7          | 50                                                                | 50                               | 0.4                |
| 9          | 10                                                                | 90                               | 0.4                |
| 10         | 5                                                                 | 95                               | 0.4                |
| 11.5       | 5                                                                 | 95                               | 0.4                |
| 11.6       | 98                                                                | 2                                | 0.4                |
| 12.5       | 98                                                                | 2                                | 0.35               |

## MS Conditions

|                     |                                                       |
|---------------------|-------------------------------------------------------|
| MS/MS System        | ABSCIEX QTrap 6500+                                   |
| Ionization Mode     | Electrospray Ionization in negative mode (-ESI-)      |
| Ion Spray Voltage   | (IS) -4500.00                                         |
| Temperature         | (TEM) 300°C                                           |
| Curtain Gas         | (CUR) 40                                              |
| Collision Gas       | (CAD) Low                                             |
| Ion Source Gas 1    | (GS1) 50                                              |
| 50 Ion Source Gas 2 | (GS2) 50                                              |
| Nitrogen Supply     | Genius 1024 LC-MS nitrogen generator, Peak Scientific |

## CHROMATOGRAMS

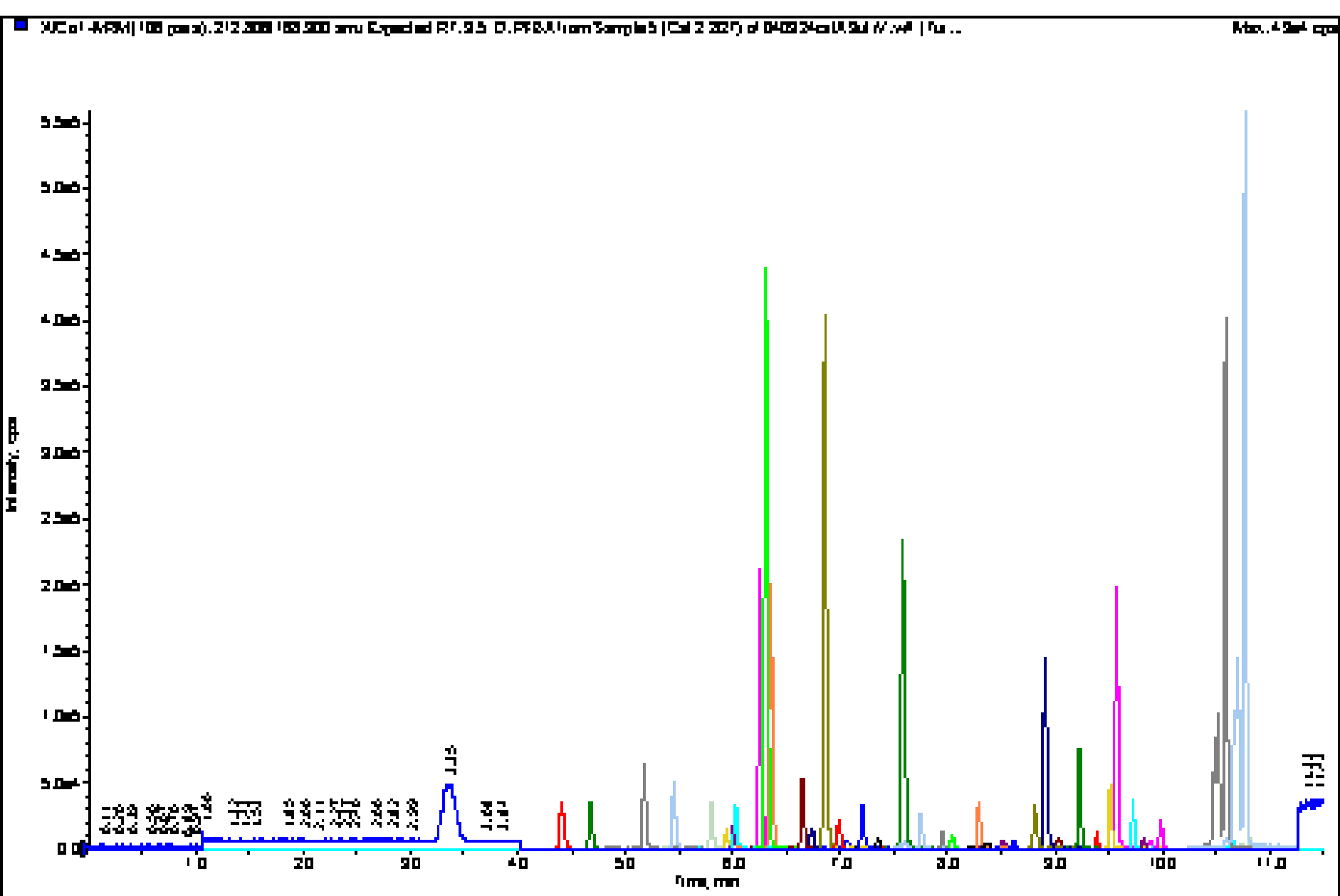


Figure 1: Analytes at 0.20 to 25.0 ng/mL

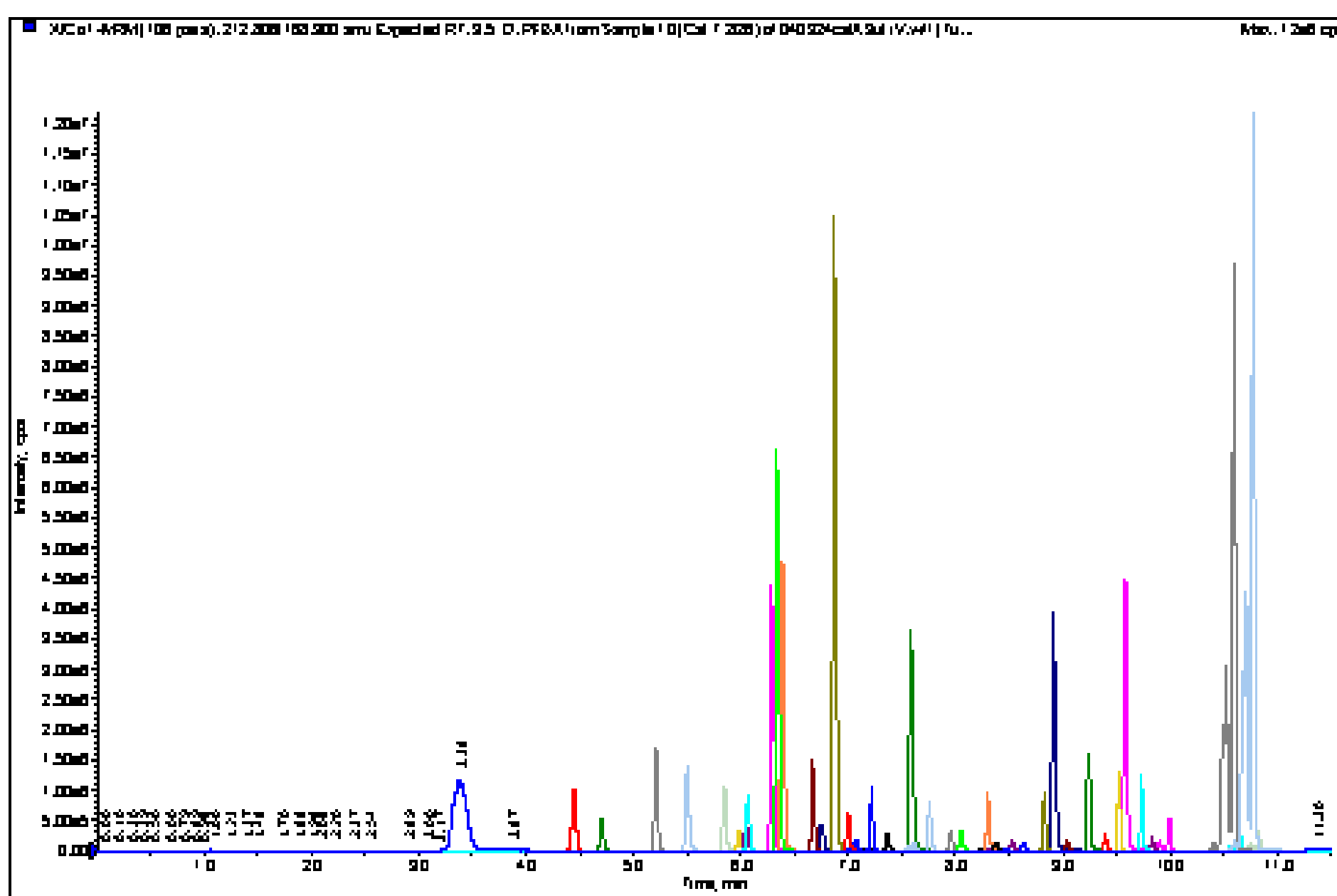


Figure 2: Analytes at 15.0 to 500 ng/mL

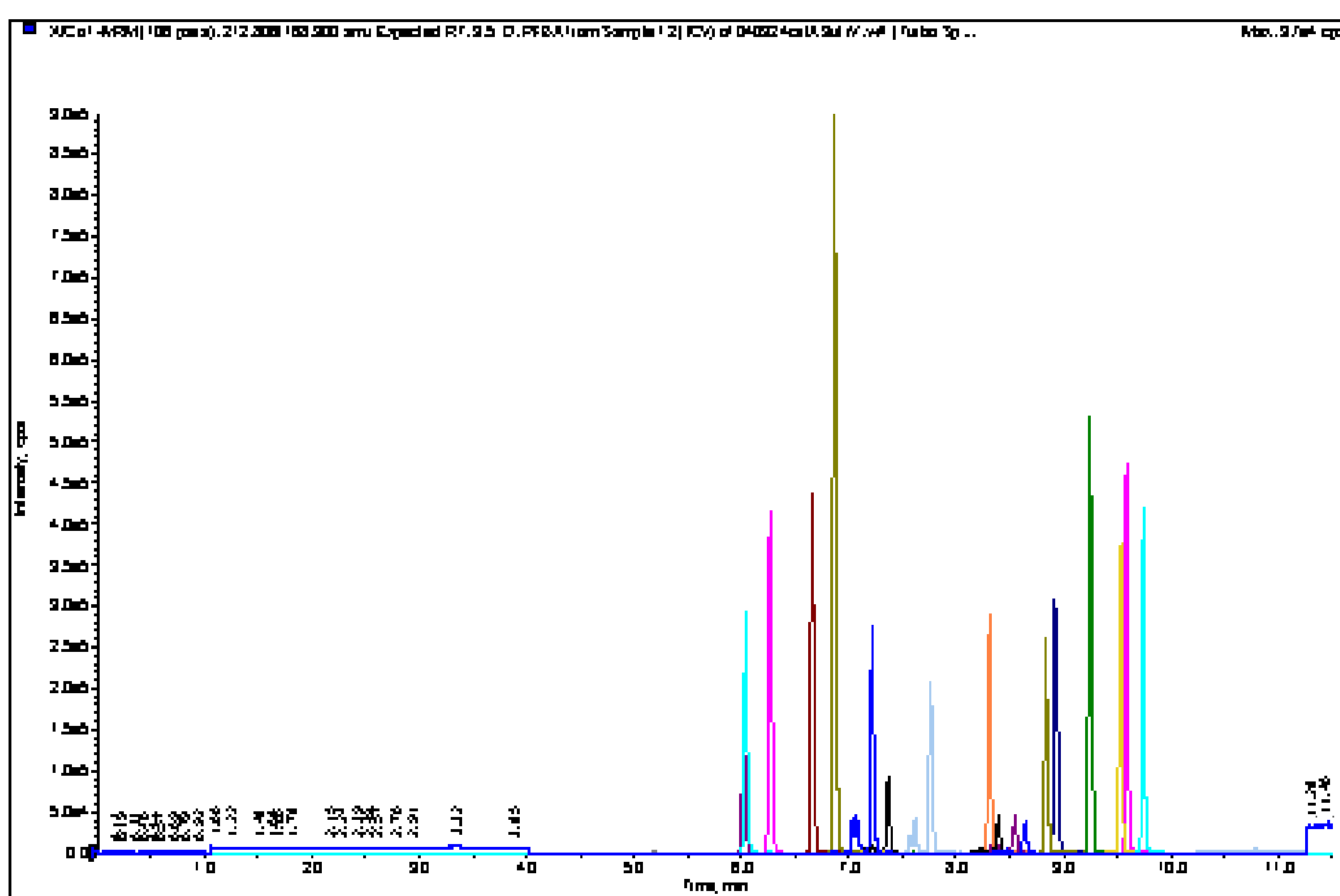


Figure 3: Initial Calibration verification of 18 PFAS at 5 ng/mL

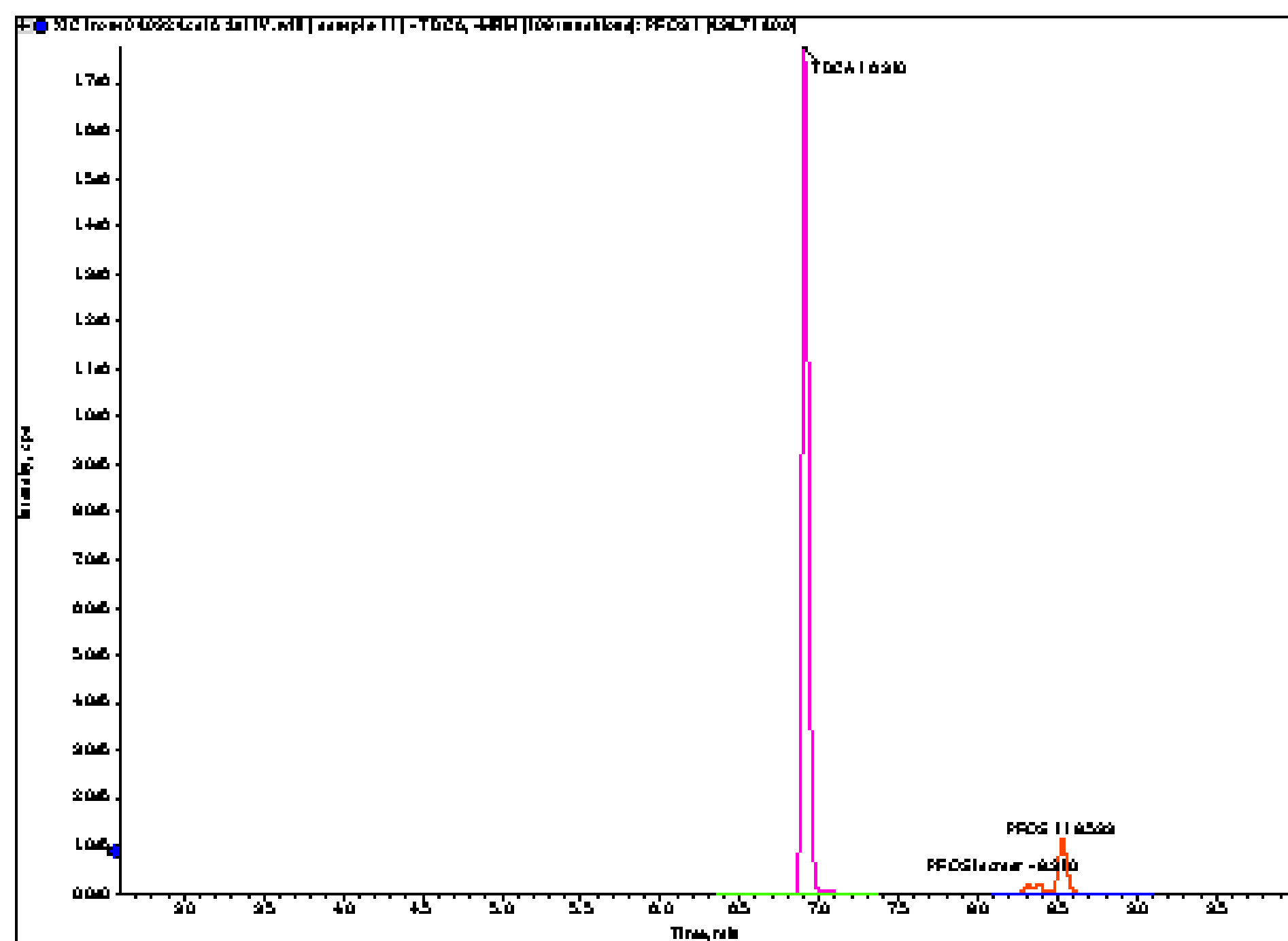


Figure 4: 1.4-minute retention time separation of TDCA and branched PFOS

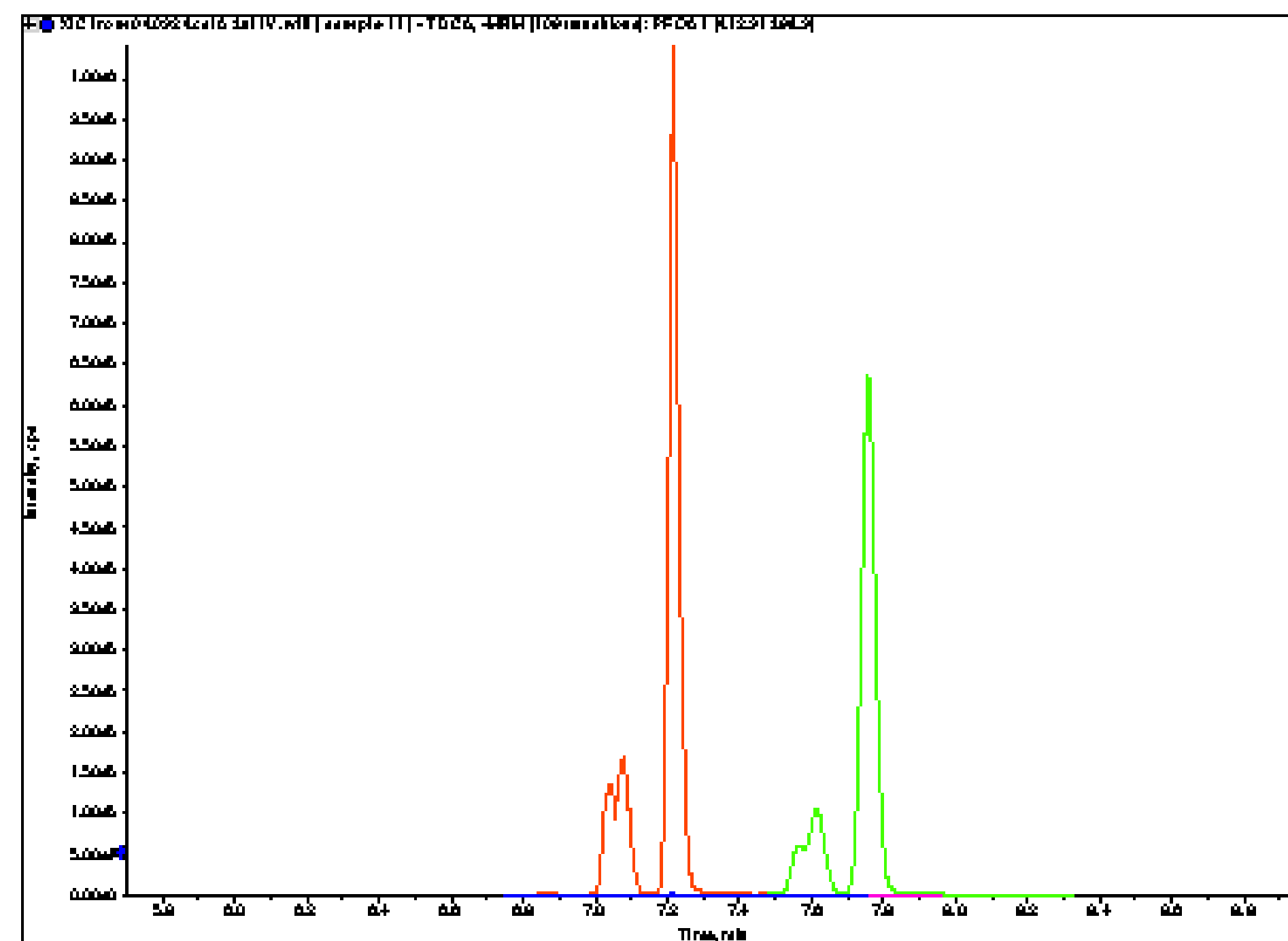


Figure 5: Isomer separation of the newly added branched PFOA and PFNA standards

## CALIBRATION CURVES

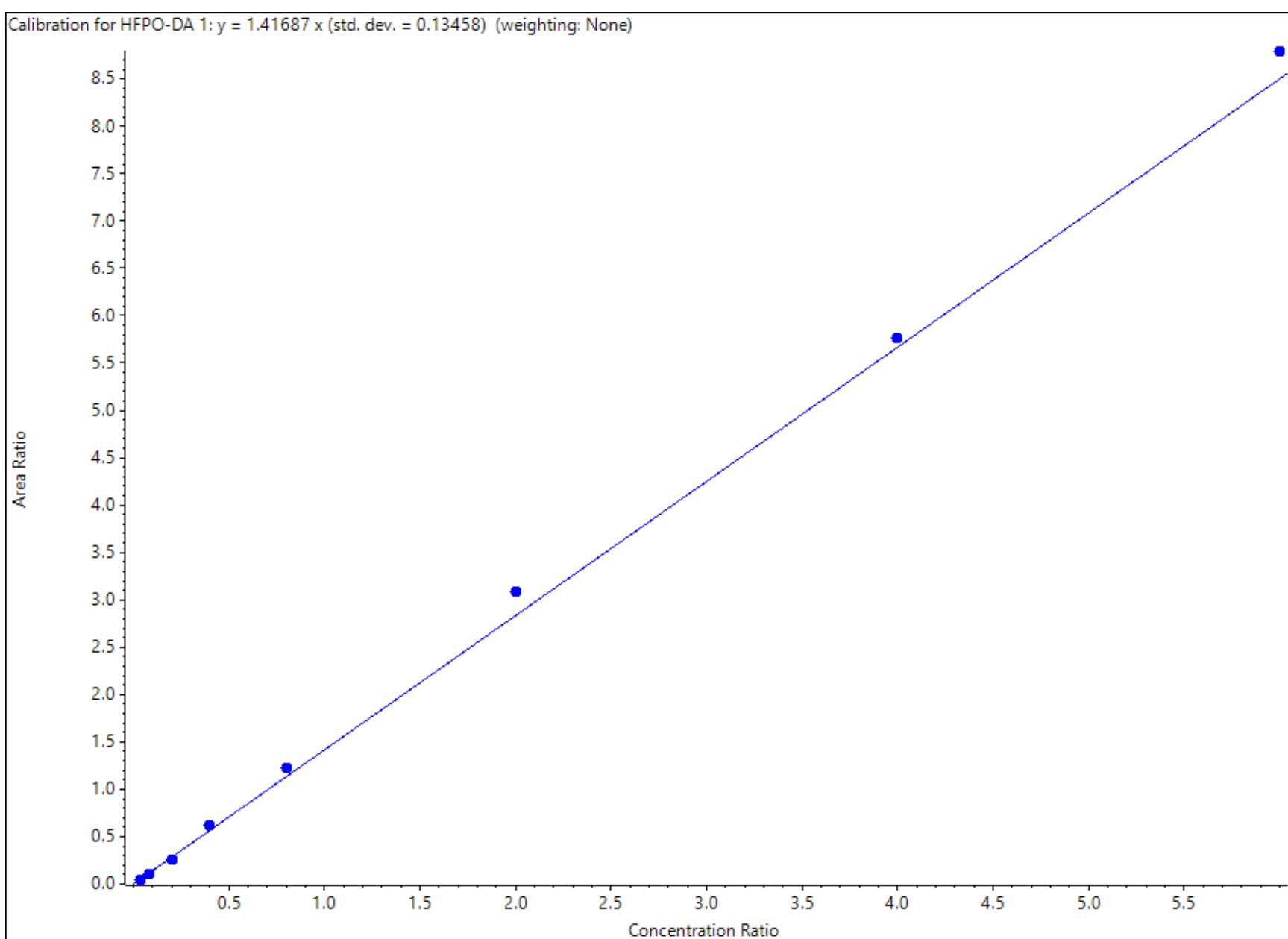


Figure 7: Calibration curve example for HFPO-DA at 0.40 to 60.0 ng/mL

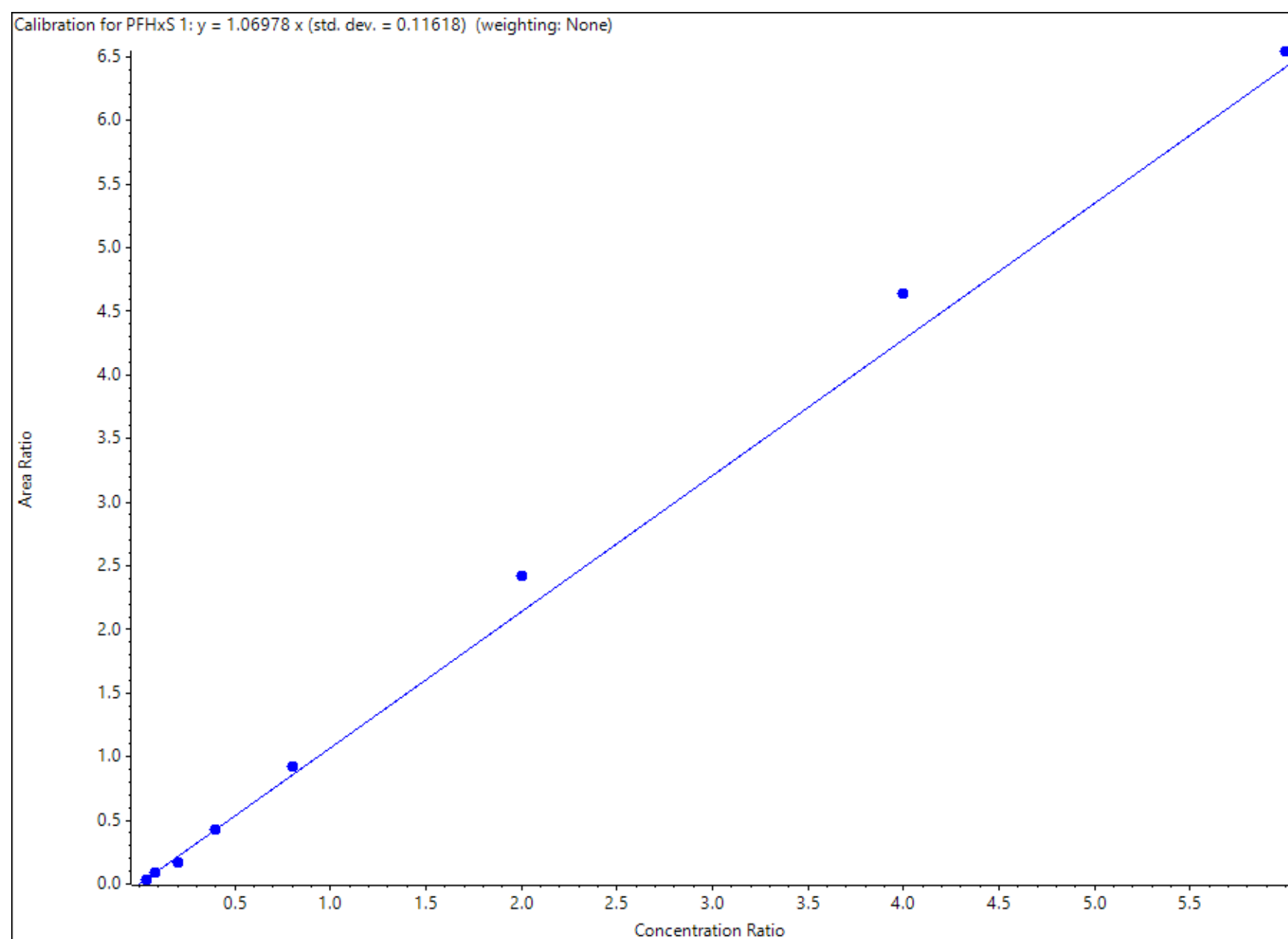


Figure 8: Calibration curve example for PFHxS at 0.09 to 13.7 ng/mL

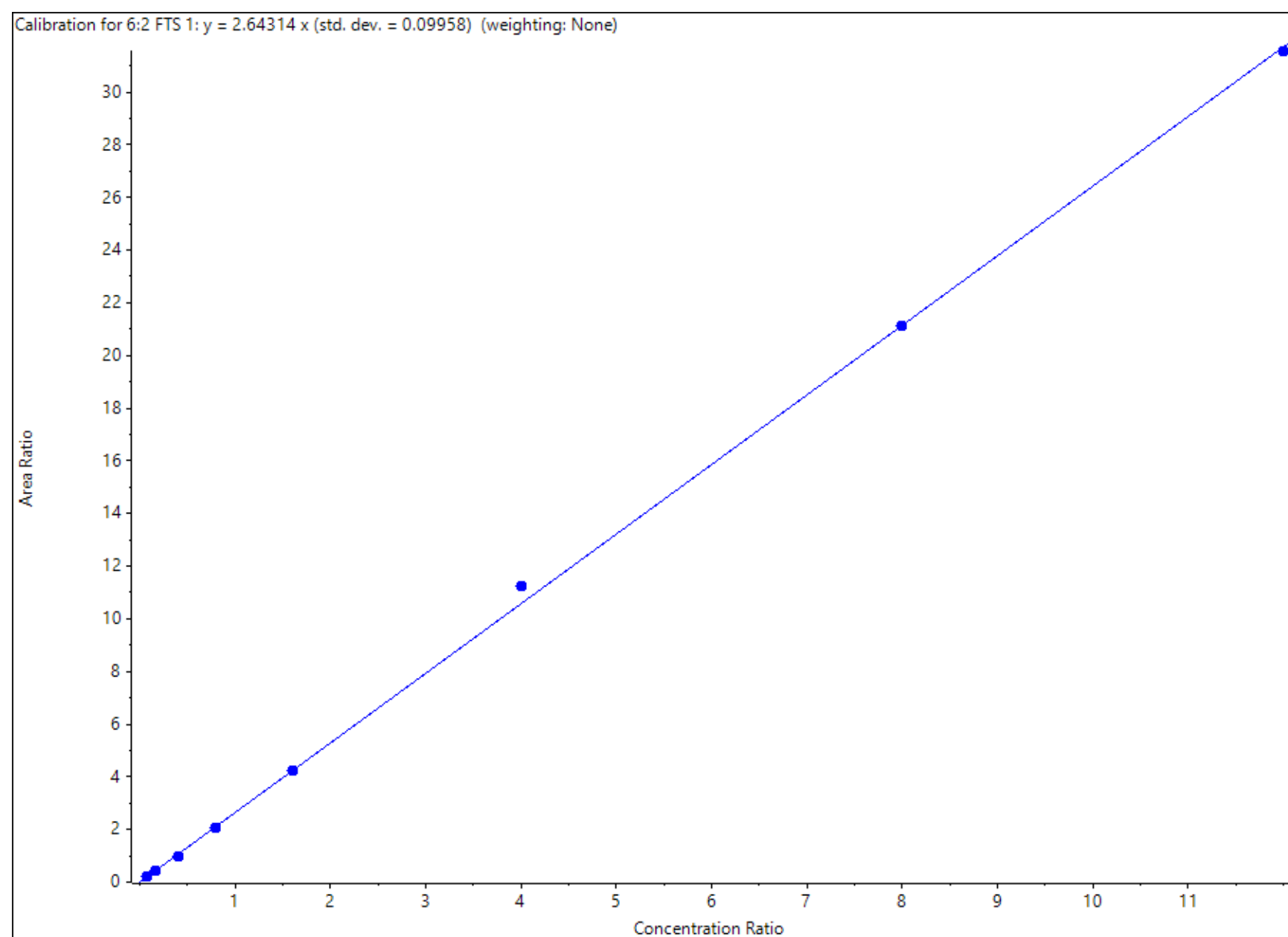


Figure 9: Calibration curve example for 6:2 FTS at 0.37 to 56.2 ng/mL

## CALIBRATION CURVE LINEARITY TABLE

| Calibration Curve Linearity |         |          |                      |      |                |
|-----------------------------|---------|----------|----------------------|------|----------------|
| Compound                    | RSD/RSE | RT (min) | Linear Range (ng/mL) |      | ICV Recovery % |
| PFBA                        | 7.74    | 3.4      | 0.40                 | 60.0 | N/A            |
| PFPeA                       | 8.41    | 5.2      | 0.20                 | 30.0 | N/A            |
| PFHxA                       | 7.49    | 6.0      | 0.10                 | 15.0 | 98             |
| PFHpA                       | 8.15    | 6.6      | 0.10                 | 15.0 | 106            |
| PFOA                        | 9.10    | 7.2      | 0.10                 | 15.0 | 98             |
| PFNA                        | 8.89    | 7.8      | 0.10                 | 15.0 | 98             |
| PFDA                        | 6.88    | 8.3      | 0.10                 | 15.0 | 93             |
| PFUnA                       | 12.01   | 8.8      | 0.10                 | 15.0 | 102            |
| PFDoA                       | 7.92    | 9.2      | 0.10                 | 15.0 | 105            |
| PFTeDA                      | 9.02    | 9.5      | 0.10                 | 15.0 | 110            |
| PFTeDA                      | 9.89    | 9.7      | 0.10                 | 15.0 | 108            |
| PFBS                        | 12.27   | 6.0      | 0.09                 | 13.3 | 94             |
| PFPeS                       | 9.67    | 6.7      | 0.09                 | 14.1 | N/A            |
| PFHxS                       | 10.86   | 7.4      | 0.09                 | 13.7 | 109            |
| PFHpS                       | 8.44    | 8.0      | 0.10                 | 14.3 | N/A            |
| PFOS                        | 12.67   | 8.5      | 0.09                 | 13.9 | 89             |
| PFNS                        | 11.62   | 9.0      | 0.10                 | 14.4 | N/A            |
| PFDS                        | 9.94    | 9.4      | 0.10                 | 14.5 | N/A            |
| PFDoS                       | 10.67   | 9.8      | 0.10                 | 14.5 | N/A            |
| 4:2 FTS                     | 8.05    | 5.8      | 0.37                 | 56.2 | N/A            |
| 6:2 FTS                     | 3.77    | 7.0      | 0.38                 | 57.1 | N/A            |
| 8:2 FTS                     | 6.89    | 8.1      | 0.38                 | 57.6 | N/A            |
| PFOSA                       | 6.60    | 10.0     | 0.10                 | 15.0 | N/A            |
| NMeFOSA                     | 8.29    | 10.7     | 0.10                 | 15.0 | N/A            |
| NetFOSA                     | 5.49    | 10.8     | 0.10                 | 15.0 | N/A            |
| NMeFOSAA                    | 7.67    | 8.4      | 0.10                 | 15.0 | 107            |
| NetFOSAA                    | 9.12    | 8.6      | 0.10                 | 15.0 | 104            |
| NMeFOSE                     | 8.83    | 10.6     | 1.00                 | 150  | N/A            |
| NetFOSE                     | 6.00    | 10.8     | 1.00                 | 150  | N/A            |
| HFPO-DA                     | 9.50    | 6.3      | 0.40                 | 60.0 | 92             |
| ADONA                       | 9.06    | 6.9      | 0.38                 | 56.7 | 104            |
| 9Cl-PF3ONS                  | 6.81    | 8.9      | 0.37                 | 56.0 | N/A            |
| 11Cl-PF3OUdS                | 8.22    | 9.6      | 0.38                 | 56.6 | 105            |
| 3:3 FTCA                    | 7.13    | 4.7      | 1.00                 | 150  | N/A            |
| 5:3 FTCA                    | 7.65    | 6.3      | 5.00                 | 500  | N/A            |
| 7:3 FTCA                    | 9.80    | 7.6      | 5.00                 | 500  | N/A            |
| PFEESA                      | 11.98   | 6.4      | 0.18                 | 26.8 | N/A            |
| PFMPA                       | 10.29   | 4.4      | 0.20                 | 30.0 | N/A            |
| PFMBA                       | 9.79    | 5.5      | 0.20                 | 30.0 | N/A            |
| NFDHA                       | 10.43   | 5.9      | 0.20                 | 30.0 | N/A            |
| TDCA                        | N/A     | 6.7      | N/A                  | N/A  | N/A            |

## CONCLUSION

UCT SelectraCore® C18 Column is made of a coreshell C18 hydrophobic phase – this technology reduces band broadening in the chromatography and backpressure on the HPLC. The results of this experiment show that the column can successfully resolve all the compounds in the method, from the more polar short-chain PFAS to the highly hydrophobic long-chain PFAS. The column showed sensitivity below the EPA's lowest suggested standard while maintaining linearity with an RSD of 80-120% for all compounds.

The column achieved a run time that was 0.5 minutes shorter than that in the EPA methodology while obtaining a 1.4-minute separation between TDCA and PFOS. The accuracy of the calibration was measured using an initial calibration verification standard at the midpoint of the calibration curve with excellent recoveries between 89-110%.