Application Note

Materials Testing and Research

# Quantitative PFAS Analysis in Medical Devices Using Triple Quadrupole LC/MS

## A reliable and sensitive analytical workflow for PFAS analysis using the Agilent 1290 Infinity III UHPLC system coupled with the Agilent 6475 LC/TQ


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## Abstract

This application note describes a comprehensive analytical workflow developed for the quantitative analysis of 73 native per- and polyfluoroalkyl substances (PFAS) in representative medical device materials, including intravenous tubing, blood collection tubes, and syringes. Samples were extracted using a streamlined methanol-based heat- and ultrasound-assisted (HUA) protocol. Analysis was performed using the Agilent 1290 Infinity III UHPLC for PFAS analysis coupled to the Agilent 6475 LC/TQ system. The UHPLC system—equipped with an Agilent 1290 Infinity III High-Speed Pump and Agilent 1290 Infinity III Hybrid Multisampler—offered ultralow system background and large-volume Feed Injection, enabling sensitive PFAS determination at low µg/kg levels in matrix. Method performance was evaluated through calibration linearity, limit of quantification (LOQ), recovery, and reproducibility, demonstrating its potential utility for research and analytical evaluation of PFAS in medical device materials.

## Introduction

Per- and polyfluoroalkyl substances (PFAS) are widely used in medical device materials due to their exceptional chemical stability, low friction, and biocompatibility. These intrinsic properties are essential for ensuring optimal performance requirements of medical device materials used in applications, including catheters, blood collection devices, contact lenses, and implantable cardiovascular systems.

Regulatory agencies worldwide are intensifying oversight of PFAS in medical devices, driven by increasing concerns over environmental persistence, potential human health impacts, and the need for improved transparency and control of PFAS use throughout the medical device life cycle. The U.S. Food and Drug Administration (FDA) states that PFAS in medical devices are subject to ongoing scientific review and regulatory evaluation, reflecting heightened oversight and scrutiny compared to previous decades.[1] North American Science Associates (NAMSA), a global Contract Research Organization focusing on medical devices, directly addresses the global regulatory pressure affecting medical device manufacturers, triggering increased monitoring, testing, and regulatory engagement.[2] Meanwhile, the Advanced Medical Technology Association (AdvaMed), the world's largest trade association for medical technology companies, highlights expanding regulatory requirements, reporting obligations and scrutiny related to PFAS use in medical devices and packaging across global markets.[3] Under the EU REACH PFAS restriction initiative (REACH 2.0), medical device applications are included within the scope of a class-based PFAS regulatory framework. While immediate bans are not foreseen for critical medical uses, regulatory oversight is intensifying, with time-limited derogations anticipated where suitable alternatives are unavailable, alongside additional controls required to minimize emissions and ensure compliance.[4,5]

So, there is an urgent need for reliable, sensitive analytical methods capable of monitoring PFAS throughout the entire medical device life cycle, from raw material qualification through finished product evaluation.

This application note presents a comprehensive analytical workflow for the screening and quantification of 73 native PFAS in medical device materials using ultrahigh-performance liquid chromatography (UHPLC) coupled with triple quadrupole mass spectrometry (LC/MS/MS). The workflow leverages the Agilent 1290 Infinity III UHPLC system for PFAS analysis, equipped with the purpose-built Agilent 1290 Infinity III High-Speed Pump and Agilent 1290 Infinity III Hybrid Multisampler, and coupled to the Agilent 6475 LC/TQ. Together, this integrated solution delivers enhanced PFAS readiness and substantially reduced background contamination, and supports sensitive and reliable PFAS monitoring in medical device research and analytical method development.

## Experimental

### Chemicals and consumables

Native and isotopically labeled PFAS standards were sourced from Wellington Laboratories Inc. (Guelph, ON, Canada) and Toronto Research Chemicals (Toronto, ON, Canada). Standards were supplied as individual stock solutions, mixed solutions, or powder forms, depending on the compound. In this work, 73 PFAS analytes, 34 surrogate standards (extracted internal standards), and three internal standards (ISTDs, non-extracted internal standards) were included.

LC/MS-grade ammonium acetate was purchased from Sigma-Aldrich (St. Louis, MO, USA). Isopropanol (IPA), Optima, was purchased from Fisher Chemical. Ultrapure LC/MS-grade acetonitrile (ACN), methanol (MeOH), and water ($H_2O$) were obtained from Agilent Technologies. Calibration standards were prepared in MeOH at concentrations ranging from 0.01 to 50 µg/L (0.01, 0.05, 0.1, 0.5, 1, 5, 10, 50 µg/L), with surrogates and ISTD added to each level at a constant concentration of 1 µg/L. All consumables used in this study were evaluated and verified for PFAS suitability following published ordering guide.[6]

## Instrumentation

Chromatographic separation was achieved on an Agilent ZORBAX RRHD Eclipse Plus C18 column (2.1 × 100 mm, 1.8 µm) installed on an Agilent 1290 Infinity III UHPLC system configured for PFAS analysis. This UHPLC system is purpose built to aggressively reduce fluorinated materials in the flow path, minimizing PFAS background contribution. The system was equipped with a 1290 Infinity III Hybrid Multisampler, enabling large-volume Feed Injection of high-organic samples. The LC pump condition and Hybrid Multisampler program are outlined in Table 1. This capability allowed direct injection of methanolic extracts while maintaining excellent peak shape and sensitivity. Analyses were performed using the Agilent 6475 LC/TQ operated in negative electrospray ionization (ESI) mode (Table 2).

**Table 1.** LC pump conditions and Hybrid Multisampler program.

<table>
<tr><th>Parameter</th><th colspan="4">LC Setting</th></tr>
<tr><td>Analytical Column</td><td colspan="4">Agilent ZORBAX RRHD Eclipse Plus C18, 95 Å, 2.1 × 100 mm, 1.8 µm,1,200 bar pressure limit (part number 959758-902)</td></tr>
<tr><td>UHPLC Guard Column</td><td colspan="4">Agilent ZORBAX RRHD Eclipse Plus C18, 2.1 mm, 1.8 µm, 1,200 bar pressure limit, UHPLC guard (part number 821725-901)</td></tr>
<tr><td>Mobile Phase A</td><td colspan="4">5 mM ammonium acetate in water</td></tr>
<tr><td>Mobile Phase B</td><td colspan="4">100% MeOH</td></tr>
<tr><td>Gradient</td><td colspan="4">Time/min %A %B Flow (mL/min)<br>0 95 5 0.4<br>1 85 15 0.4<br>1.5 45 55 0.4<br>5.5 30 70 0.4<br>7 20 80 0.4<br>12 0 100 0.4<br>14.4 0 100 0.4<br>14.5 85 15 0.4</td></tr>
<tr><td>Stop Time</td><td colspan="4">14.5 minutes</td></tr>
<tr><td>Post-Time</td><td colspan="4">2.5 minutes</td></tr>
<tr><th colspan="5">Hybrid Multisampler Program</th></tr>
<tr><td>Injection Mode</td><td colspan="4">Feed</td></tr>
<tr><td>Injection Volume</td><td colspan="4">30 µL</td></tr>
<tr><td>Feed Speed</td><td colspan="4">Adaptive, 10% of pump flow</td></tr>
<tr><td>Flush Out Mode</td><td colspan="4">Automatic</td></tr>
<tr><td>Injection Path Cleaning</td><td colspan="4">Inner wash mode: Extended<br>Outer wash mode: Extended<br>
<table>
<tr><th>Step</th><th>Task</th><th>Solution</th><th>Duration/ Volume</th></tr>
<tr><td colspan="4">Draw Sample</td></tr>
<tr><td>1</td><td>Outer wash</td><td>S1 = 50:50 ACN:IPA</td><td>15 s</td></tr>
<tr><td>2</td><td>Outer wash</td><td>S3 = 100% MeOH</td><td>15 s</td></tr>
<tr><td colspan="4">Injection</td></tr>
<tr><td>1</td><td>Inner wash</td><td>S1 = 50:50 ACN:IPA</td><td>150 µL</td></tr>
<tr><td>2</td><td>Inner wash</td><td>S3 = 100% MeOH</td><td>150 µL</td></tr>
<tr><td>3</td><td>Seat wash</td><td>S1 = 50:50 ACN:IPA</td><td>150 µL</td></tr>
<tr><td>4</td><td>Seat wash</td><td>S3 = 100% MeOH</td><td>150 µL</td></tr>
<tr><td>5</td><td>Reconditioning</td><td>S2 = 95:5 MPA:MPB</td><td></td></tr>
</table></td></tr>
</table>

**Table 2.** Agilent 6475 LC/TQ parameters.

| 6475 LC/TQ Parameters | |
|---|---|
| Ion Source | Agilent Jet Stream (AJS) ESI |
| Polarity | Negative |
| Q1 and Q3 Resolution | Unit |
| Cycle Time | 580 ms |
| Gas Temperature | 230 °C |
| Gas Flow | 6 L/min |
| Nebulizer | 20 psi |
| Sheath Gas Temperature | 375 °C |
| Sheath Gas Flow | 12 L/min |
| Capillary (Negative/Positive) | 2,500 V |
| Nozzle Voltage | 0 V |

### Sample preparation

Medical device samples (intravenous tubing, blood collection tubes, and syringes) were sourced locally. Intravenous tubing was designated as the quality control (QC) sample to evaluate workflow performance. Blood collection tubes and syringes were analyzed as unknown samples to assess method applicability for PFAS screening in matrix. All samples were cut into small pieces (< 1 $cm^2$ per piece) by industrial stainless-steel scissors. Figure 1 illustrates the solvent-based sample extraction procedure in detail.[7]

Over the entire sample preparation process, a 10-fold dilution was induced prior to analysis. Matrix-spiked QC samples were prepared at three concentration levels: 1 µg/kg (LSQ: low-spiked QC, equivalent to 0.1 µg/kg in extract), 10 µg/kg (MSQ: medium-spiked QC, equivalent to 1 µg/kg in extract), and 100 µg/kg (HSQ: high-spiked QC, equivalent to 10 µg/kg in extract). Each QC level was prepared in triplicate to evaluate method limits of quantification (LOQs), recovery, and reproducibility.

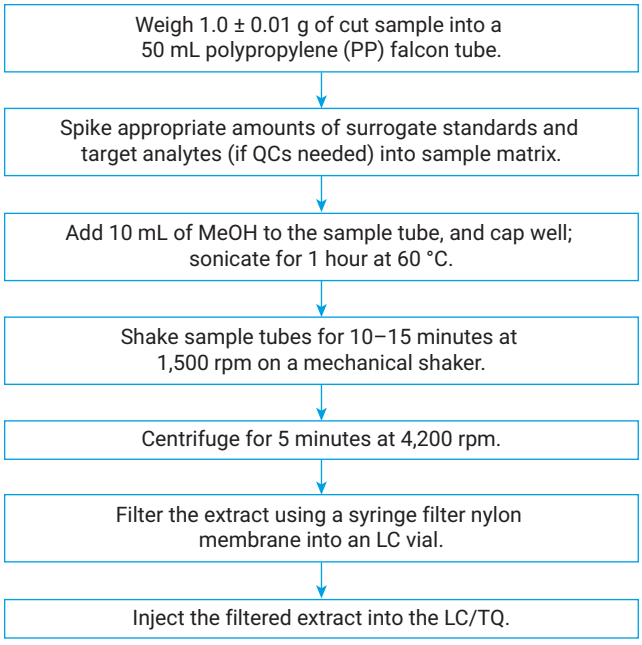


**Figure 1.** Sample preparation procedure for medical device materials.

## Results and discussion

### Ultralow PFAS background

Achieving ultralow background levels is critical for PFAS analysis, as contamination introduced by the analytical system, solvents, or sample preparation steps can directly impact method sensitivity. Figure 2 presents a total ion chromatogram (TIC) overlay of the procedural blank and calibration standard 1 (Cal 1, 0.01 µg/L). The procedural blank exhibits negligible PFAS background signals. These results demonstrate that the 1290 Infinity III UHPLC system, purpose-built for PFAS analysis, effectively minimizes background contamination from fluorinated materials in the flow and is therefore well suited for ultratrace-level PFAS analysis.

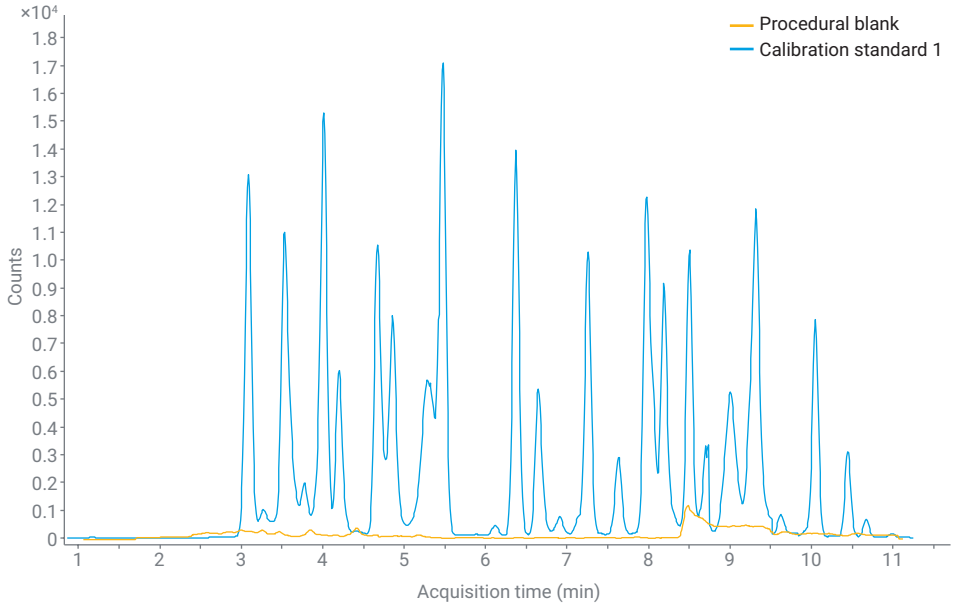


**Figure 2.** TIC overlay of procedural blank and calibration standard 1 with 30 µL Feed Injection.

## Feed Injection optimization

For PFAS analysis, the UHPLC was equipped with the 1290 Infinity III Hybrid Multisampler, enabling Feed Injection of a large-volume test solution in 100% organic solvent. In this study, calibration standards and sample extract were prepared in MeOH. Optimization of injection volume and feed speed revealed that a 30 µL injection volume with a feed speed of 10% of the pump flow rate (adaptive) delivered good peak shape and maximum abundance for all analytes. Figures 3 and 4 show chromatograms obtained with different injection volumes for early-eluting compounds (PFBA and PFHxS) and late-eluting compounds (PFHxDA and PFODA). This optimization ensured consistent chromatographic performance across the full range of 73 PFAS analytes separated within a 12-minute gradient window using a ZORBAX RRHD Eclipse Plus C18 column.

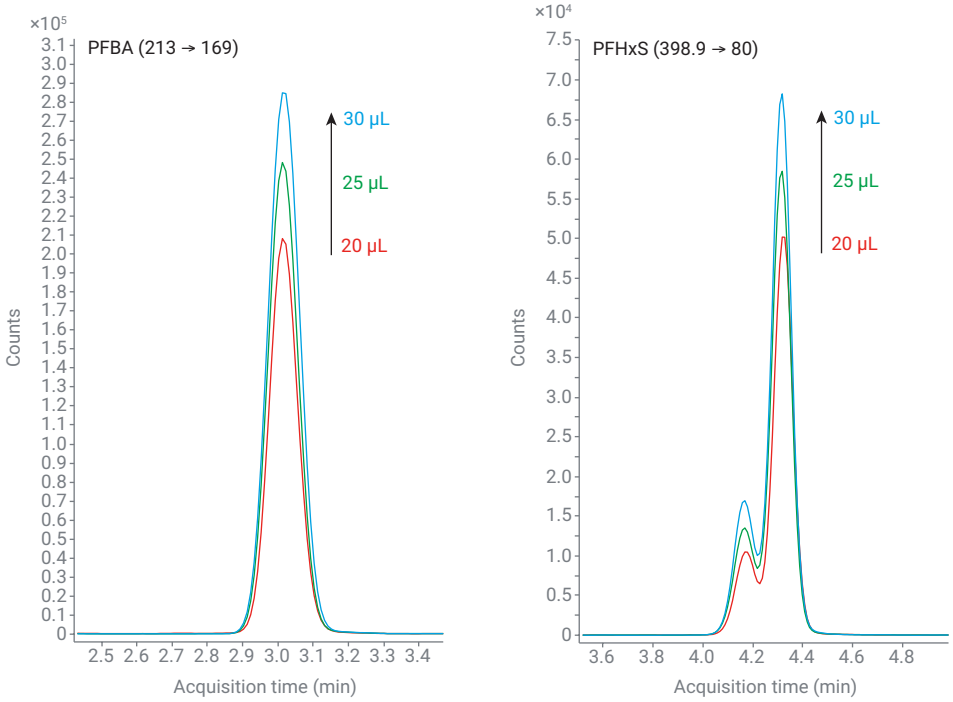


**Figure 3.** Feed Injection volume optimization for the early-eluting compounds PFBA and PFHxS.

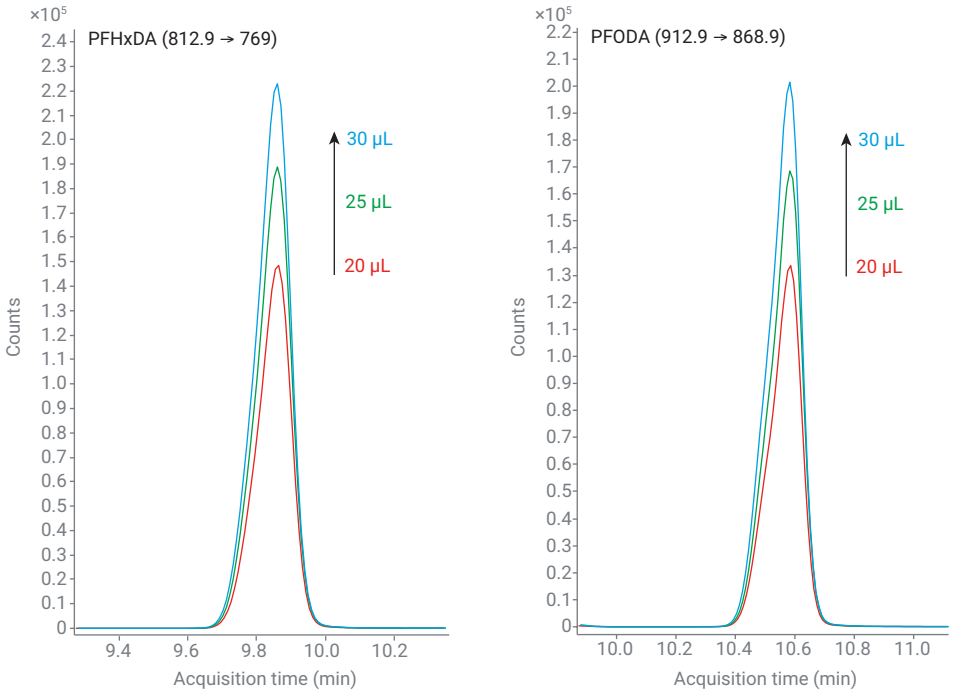


**Figure 4.** Feed Injection volume optimization for the late-eluting compounds PFHxDA and PFODA.

## Calibration performance and method sensitivity

All 73 PFAS analytes demonstrate excellent linearity across a minimum of five calibration levels, with $R^2$ values exceeding 0.992. Representative calibration curves for four key regulatory compounds—PFOA, PFNA, PFOS, and PFHxS—confirm robust quantitative performance (Figure 5).

Method LOQs were established based on matrix-spiked QC levels (LSQ, MSQ, and HSQ) meeting 65–135% recovery and fulfilling compound identification criteria, including signal-to-noise ratio (S/N) and ion ratio. Method LOQs are summarized in Table 3. Of the 73 target analytes, 65 achieved LOQs at 1 µg/kg, including all 40 mandatory PFAS compounds listed in EPA Method 1633 for environmental matrices (highlighted in blue).[8] These results demonstrate the high sensitivity of the PFAS-ready 6475 LC/TQ system, which enables precise quantification of PFAS at ultratrace levels in medical device materials. Lower limit of quantification (LLOQ) in neat solvent was defined for 6:8 PFPi and 8:8 PFPi due to consistent poor recoveries observed across all QCs in this study.

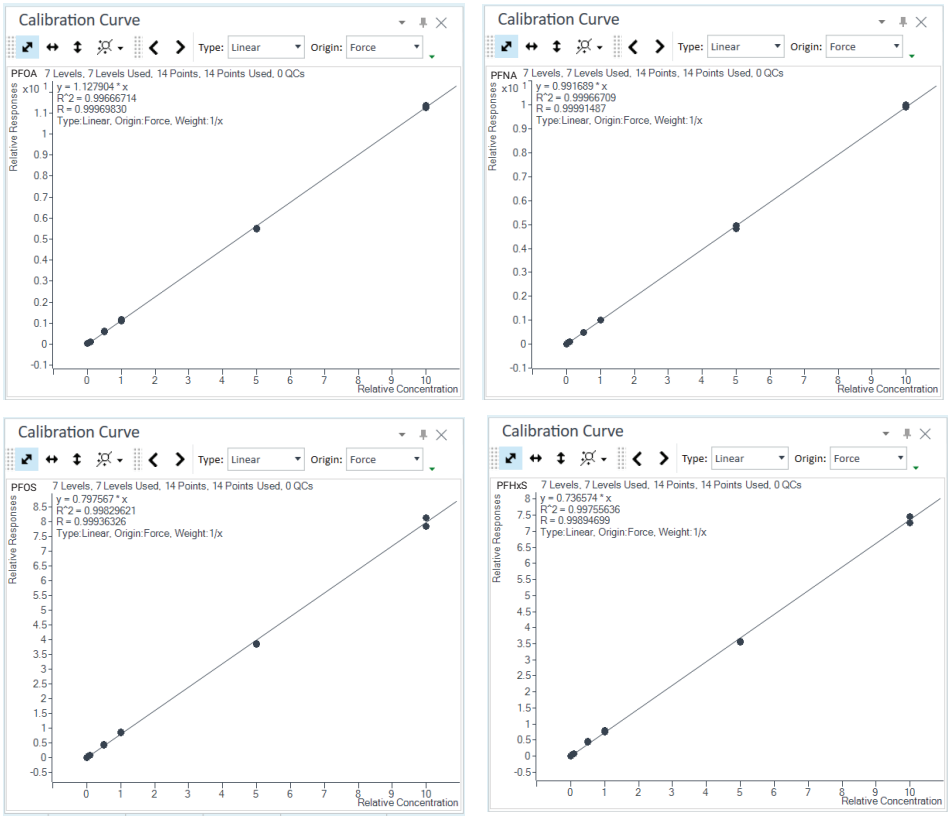


**Figure 5.** Representative calibration curves for four key regulatory compounds—PFOA, PFNA, PFOS, and PFHxS.

**Table 3.** Summary of target LOQs and QC recoveries.

| No. | Compound Name | CAS No. | LOQ (µg/kg) | Recovery (%) | | |
|---|---|---|---|---|---|---|
| | | | | LSQ | MSQ | HSQ |
| 1 | PFBPA | 52299-24-8 | 10 | 141 | 135 | 131 |
| 2 | PFBA | 375-22-4 | 1 | 127 | 111 | 102 |
| 3 | PFMPA | 377-73-1 | 1 | 103 | 106 | 102 |
| 4 | PFPeA | 2706-90-3 | 1 | 100 | 102 | 103 |
| 5 | 3:3 FTCA | 356-02-5 | 1 | 110 | 104 | 102 |
| 6 | PFBS | 375-73-5 | 1 | 104 | 104 | 103 |
| 7 | PFHxPA | 40143-76-8 | 100 | 164 | 136 | 116 |
| 8 | PFMBA | 863090-89-5 | 1 | 98 | 103 | 101 |
| 9 | Cl-PFHxPA | N/A | 100 | 185 | 172 | 156 |
| 10 | PFEESA | 113507-82-7 | 1 | 108 | 111 | 111 |
| 11 | NFDHA | 151772-58-6 | 1 | 99 | 100 | 99 |
| 12 | 4:2 FTSA | 757124-72-4 | 1 | 99 | 99 | 69 |
| 13 | PFHxA | 307-24-4 | 1 | 104 | 102 | 101 |
| 14 | PFPeS | 2706-91-4 | 1 | 96 | 101 | 97 |
| 15 | HFPO-DA | 13252-13-6 | 1 | 105 | 108 | 106 |
| 16 | FBSA | 30334-69-1 | 1 | 101 | 105 | 103 |
| 17 | P5MeODIOXOAc | 1190931-41-9 | 1 | 119 | 115 | 108 |
| 18 | PFHpA | 375-85-9 | 1 | 101 | 99 | 99 |
| 19 | PFHxS | 355-46-4 | 1 | 102 | 108 | 106 |

**Table 3.** Summary of target LOQs and QC recoveries (continued).

| No. | Compound Name | CAS No. | LOQ (µg/kg) | Recovery (%) | | |
|---|---|---|---|---|---|---|
| | | | | LSQ | MSQ | HSQ |
| 20 | DONA | 919005-14-4 | 1 | 91 | 92 | 94 |
| 21 | PFOPA | 40143-78-0 | 1 | 124 | 104 | 102 |
| 22 | 5:3 FTCA | 914637-49-3 | 1 | 104 | 105 | 106 |
| 23 | 6:2 FTUCA | 70887-88-6 | 1 | 102 | 113 | 113 |
| 24 | 6:2 FTCA | 53826-12-3 | 1 | 98 | 99 | 99 |
| 25 | 4-PFecHS | 646-83-3 | 1 | 83 | 91 | 94 |
| 26 | 6:2 FTSA | 27619-97-2 | 1 | 98 | 95 | 92 |
| 27 | PFOA | 335-67-1 | 1 | 134 | 101 | 99 |
| 28 | PFHpS | 375-92-8 | 1 | 95 | 96 | 96 |
| 29 | MeFBSA | 68298-12-4 | 1 | 94 | 98 | 100 |
| 30 | FHxSA | 41997-13-1 | 1 | 92 | 97 | 95 |
| 31 | PFNA | 375-95-1 | 1 | 99 | 101 | 103 |
| 32 | PFOS | 1763-23-1 | 1 | 97 | 100 | 99 |
| 33 | 8:2 FTUCA | 70887-84-2 | 1 | 89 | 92 | 93 |
| 34 | PFDPA | 52299-26-0 | 10 | 180 | 135 | 127 |
| 35 | 7:3 FTCA | 812-70-4 | 1 | 95 | 96 | 97 |
| 36 | HFPO-TA | 13252-14-7 | 1 | 97 | 94 | 95 |
| 37 | 8:2 FTCA | 27854-31-5 | 100 | 503 | 137 | 101 |
| 38 | 9Cl-PF3ONS | 756426-58-1 | 1 | 94 | 95 | 97 |
| 39 | FOSAA | 2806-24-8 | 1 | 90 | 94 | 92 |
| 40 | 8:2 FTSA | 39108-34-4 | 1 | 98 | 95 | 70 |
| 41 | PFNS | 68259-12-1 | 1 | 102 | 102 | 101 |
| 42 | PFDA | 335-76-2 | 1 | 97 | 101 | 103 |
| 43 | 8:3 FTCA | 34598-33-9 | 1 | 94 | 104 | 108 |
| 44 | N-MeFOSAA | 2355-31-9 | 1 | 94 | 102 | 100 |
| 45 | MeFHxSA | 68259-15-4 | 1 | 93 | 95 | 95 |
| 46 | PFDS | 335-77-3 | 1 | 98 | 99 | 99 |
| 47 | PFUnDA | 2058-94-8 | 1 | 85 | 90 | 91 |
| 48 | N-EtFOSAA | 2991-50-6 | 1 | 90 | 100 | 105 |
| 49 | PFOSA | 754-91-6 | 1 | 96 | 102 | 102 |
| 50 | 10:2 FTUCA | 70887-94-4 | 1 | 105 | 107 | 107 |
| 51 | 11Cl-PF3OUdS | 763051-92-9 | 1 | 90 | 94 | 96 |
| 52 | PFUnDs | 749786-16-1 | 1 | 84 | 85 | 88 |
| 53 | PFDoDA | 307-55-1 | 1 | 101 | 105 | 104 |
| 54 | 10:2 FTSA | 120226-60-0 | 1 | 104 | 104 | 80 |
| 55 | 10:2 FTCA | 53826-13-4 | 100 | 495 | 136 | 100 |
| 56 | 6:6 PFPi | 40143-77-9 | 1 | 76 | 83 | 85 |
| 57 | PFDoS | 79780-39-5 | 1 | 96 | 99 | 97 |
| 58 | PFTrDA | 72629-94-8 | 1 | 91 | 96 | 97 |
| 59 | N-MeFOSA | 31506-32-8 | 1 | 97 | 96 | 97 |
| 60 | FDSA | N/A | 1 | 106 | 112 | 108 |
| 61 | MeFOSE | 24448-09-7 | 1 | 98 | 102 | 98 |
| 62 | PFTrDS | 791563-89-8 | 1 | 89 | 90 | 89 |
| 63 | 6:2 diPAP | 57677-95-9 | 1 | 104 | 106 | 106 |
| 64 | PFTDA | 376-06-7 | 1 | 96 | 100 | 101 |

**Table 3.** Summary of target LOQs and QC recoveries (continued).

| No. | Compound Name | CAS No. | LOQ (µg/kg) | Recovery (%) | | |
|---|---|---|---|---|---|---|
| | | | | LSQ | MSQ | HSQ |
| 65 | 6:8 PFPi | 610800-34-5 | 10 (ng/L)* | 36 | 38 | 40 |
| 66 | N-EtFOSA | 4151-50-2 | 1 | 91 | 120 | 122 |
| 67 | EtFOSE | 1691-99-2 | 1 | 109 | 103 | 105 |
| 68 | 6:2/8:2 diPAP | 943913-15-3 | 1 | 75 | 74 | 76 |
| 69 | 8:8 PFPi | 40143-79-1 | 10 (ng/L)* | 35 | 38 | 39 |
| 70 | PFHxDA | 67905-19-5 | 1 | 105 | 104 | 103 |
| 71 | 8:2 diPAP | 678-41-1 | 1 | 96 | 101 | 99 |
| 72 | PFODA | 16517-11-6 | 1 | 96 | 100 | 101 |
| 73 | diSAmPAP | 2965-52-8 | 1 | 106 | 104 | 117 |

*LLOQ was defined.

## QC recovery and reproducibility

Matrix-spiked QC recovery was used to assess the extraction efficiency of a sample preparation protocol based on methanol extraction followed by heat- and ultrasound-assisted treatment. Over 87% of analytes demonstrate recoveries within the acceptable 65–135% range at all QC levels (Table 3), indicating high extraction efficiency across diverse PFAS chemical classes.

Method reproducibility was evaluated based on the relative standard deviation (%RSD) of QC recoveries at each QC level (n = 6, three technical preparations with two injections per preparation). All 73 analytes exhibit %RSD values below 20% across LSQ, MSQ, and HSQ levels, demonstrating excellent reproducibility of the end-to-end analytical workflow.

Figure 6 shows %RSD ≤ 13 for 20 representative PFAS compounds of key concern in other industries, such as textile manufacturing[9], including PFOA, PFOS, PFNA, and PFHxS. This reproducibility is essential for routine PFAS monitoring in medical device materials and supports regulatory compliance applications.

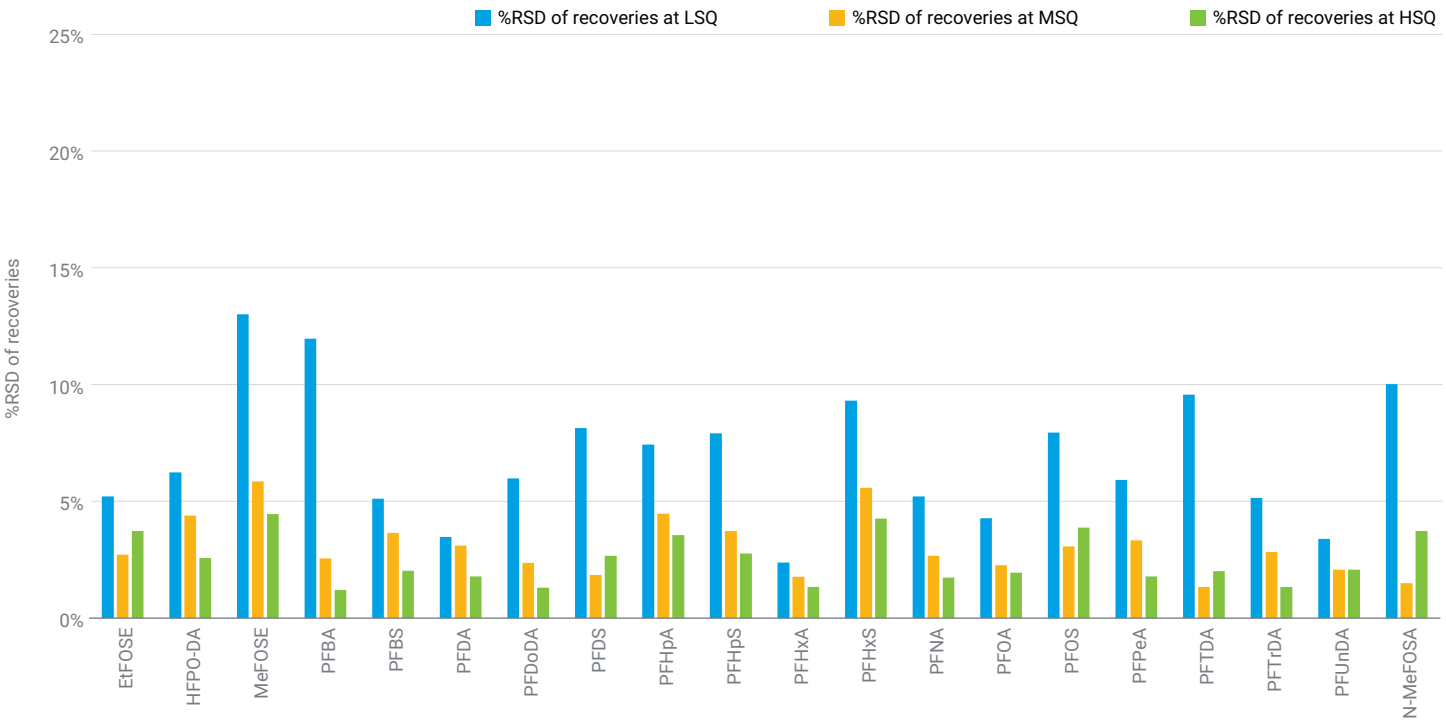


**Figure 6.** %RSDs of recoveries for the representative 20 PFAS compounds across all QC levels.

## Sample analysis

To validate the applicability and reliability of the workflow for real-world medical device materials, blood collection tubes (S1) and syringes (S2) were taken as unknown samples in this study. Sample extracts of S1 and S2 in duplicate confirmed trace amounts of PFOA above the method detection limit (MDL; Figures 7A and 7B). This data provides useful insight for medical device manufacturers monitoring PFAS in raw materials or finished products, supporting quality assurance and regulatory compliance.

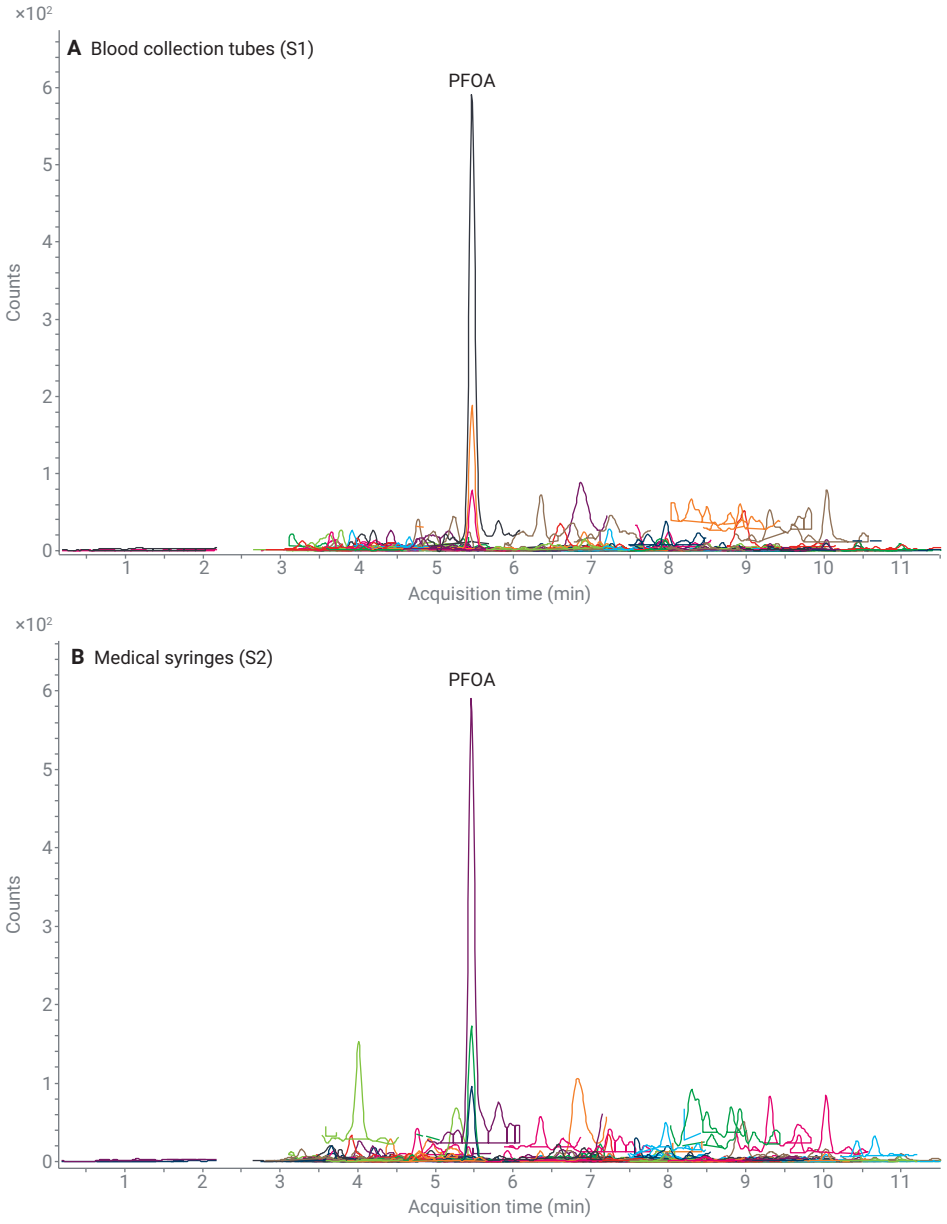


**Figure 7.** The multiple reaction monitoring (MRM) chromatograms of unspiked sample extracts (A) S1 and (B) S2.

## Conclusion

This application note demonstrates a comprehensive analytical workflow for quantitative PFAS analysis in medical device materials using an Agilent 1290 Infinity III UHPLC system for PFAS analysis coupled with an Agilent 6475 LC/TQ. The purpose-built system, featuring an Agilent 1290 Infinity III High-Speed Pump and 1290 Infinity III Hybrid Multisampler, achieved ultralow background contamination and exceptional sensitivity for 73 native PFAS analytes. Method reproducibility was excellent, with all analytes showing %RSD values below 20% across all QC levels, providing reliable data for regulatory compliance in medical device manufacturing. This integrated approach combines advanced instrumentation with optimized method parameters to establish a robust platform for PFAS monitoring throughout the medical device life cycle, supporting manufacturers in meeting evolving regulatory requirements while ensuring product safety and environmental stewardship.

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