Application

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ePrep | Application Note 2025

ePrep Automated Preparation for calibration standards for Per- and polyfluoroalkyl substances (PFAS)

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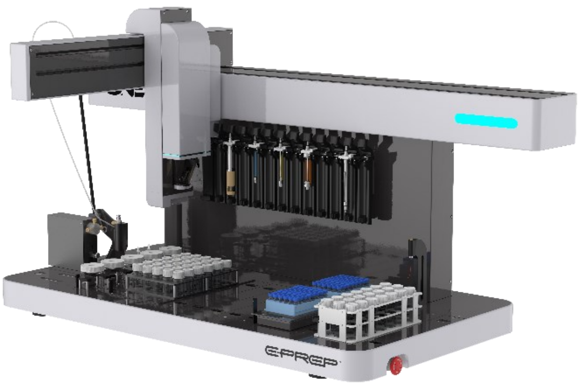


Figure 1 *ePrep Sample Preparation Workstation*

SUMMARY

The ePrep ONE automates preparation of a PFAS calibration standard curve series using optimized Workflow tasks. A major advantage of ePrep is its ability to prepare standards at small volumes (<1.5mL) with high accuracy and precision. This reduces the use of expensive standards whilst eliminating errors carried through from large volume preparation. ePrep demonstrates excellent linearity across a range of prepared PFAS compound classes. No manual intervention is required, which eliminates the requirements of high skilled labour for standards preparation. This Application Note prepares instrument (LC/MS/MS) Calibration Standards at a 50 to 5000 ng/L range with 500 ng/L Internal Standard specified for ASTM 8421.

INTRODUCTION

PFAS compounds, distinguished by their unique fluorinated hydrocarbon chains bonded to charged functional groups, have become ubiquitous in modern life through their extensive use in commercial and industrial applications. From protective fabric coatings to non-stick cookware and specialized firefighting foams, PFAS compounds deliver exceptional performance characteristics that have made them indispensable across numerous sectors. However, their remarkable stability—earning them the designation "forever chemicals"—has led to widespread environmental persistence, creating urgent demands for precise analytical methodologies

The accuracy and reliability of analytical methods depend critically on well-prepared calibration standards that serve as reference points for instrument calibration and method validation. Modern analytical techniques, particularly LC-MS/MS and GC-MS/MS, require calibration standards at increasingly lower concentrations, demanding precise handling of small volumes across multiple dilution steps.

Creating calibration standards presents several challenges for laboratories. When commercial standards are unavailable, analysts must precisely combine numerous reference materials and perform serial dilutions to achieve the required concentrations. This process becomes particularly complex for multi-component mixtures that may contain dozens or hundreds of analytes, requiring significant time and attention to detail. Manual preparation of calibration standards demands precision and accuracy, yet performing these repetitive dilutions manually can impact even skilled analysts' performance and is time consuming. It is labour intensive, handling small volumes accurately requires significant skill and proper techniques to maintain precision. Operator variability and manual pipetting inconsistencies does make this process challenging.

The ePrep ONE workstation addresses these challenges through automated liquid handling designed specifically for chromatographic applications. While calibration standard preparation represents just one of its capabilities, the system's offline operation allows preparing stock solutions and calibrants while maintaining availability for other analytical workflows. By eliminating manual errors in dilution steps and reducing hands-on time for standard preparation, it optimizes resource allocation while maintaining improved calibration curve linearity.

INSTRUMENTATION

Table 1 LC parameters

Parameter	Value
Model:	6495 triple Quadrupole LCMS/MS
Column temp:	45°C
Column:	Aq-C18 4.6x100mm P/N 695975-742
Flowrate:	0.5 ml/min
Injection vol:	5 µL
Mobile phase A:	5 mM ammonium acetate in ultra-pure water
Mobile phase B:	Methanol (LCMS) grade
Gradient:	17-minute total runtime

Time (min)	A. Conc (%)	B. Conc (%)
0	80	20
0.5	80	20
2.5	50	50
7	28	72
10	10	90
14.40	10	90
14.41	80	20

16	80	20
16.05	80	20

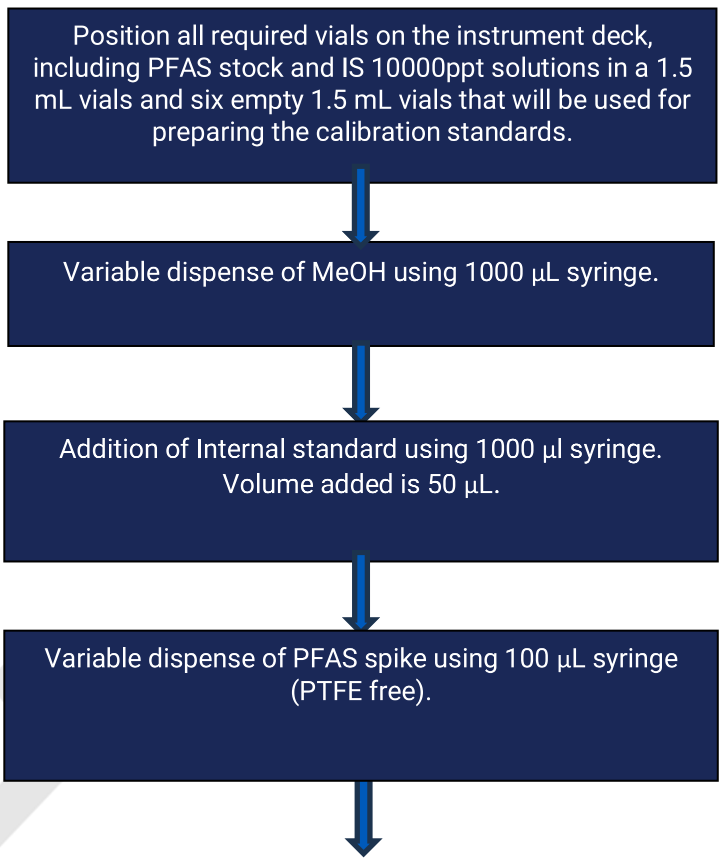
Table 2 MS parameters

Parameter	Value
Model:	Agilent 6495D
Ionising mode:	Negative
Sheath Gas Flow:	12 L/min
Nebulizer Pressure:	30psi
Capillary Voltage:	-2350 V
Gas Temperature:	250°C

WORKFLOW

The ePrep software is a task-based user interface. No movements need to be programmed, the groups of vials are defined, and the tasks are selected. Within the ePrep software there are several tasks that allow for easy use of the system for creation of calibration standards. These include variable dispense, serial dilution, serial dispense and make up to volume. Mixing to homogenise the final solution can be done through a syringe mix process or using a vortex shaker that can be placed on a deck. Wash Steps automatically exist within the tasks which can use an in-built Wash Station or Deck Vials. Wash steps can be adjusted to increase volume and cycles for sticky PFAS analytes.

The Workflow for preparation of PFAS calibration standards is shown below.



Variable dispense of PFAS spike using 1000 μL syringe (PTFE free).

Vortex using 2 pin vortex mixer for 60 seconds at 1100 RPM.

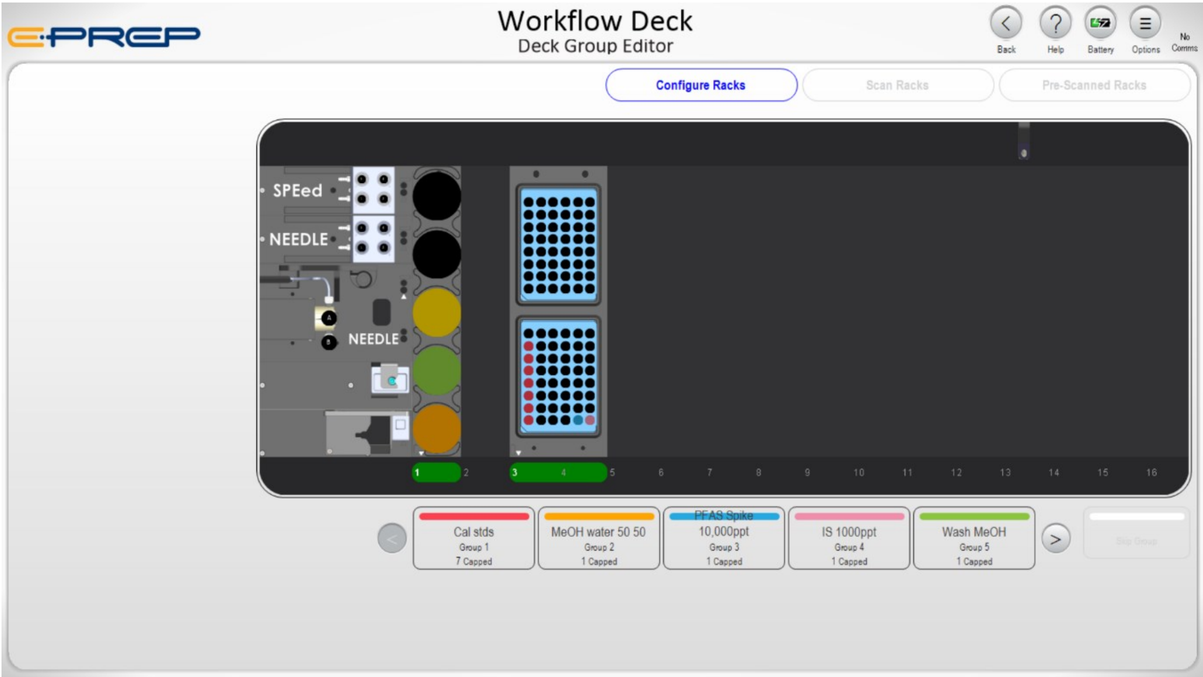


Figure 2 Deck display showing vial set-up.

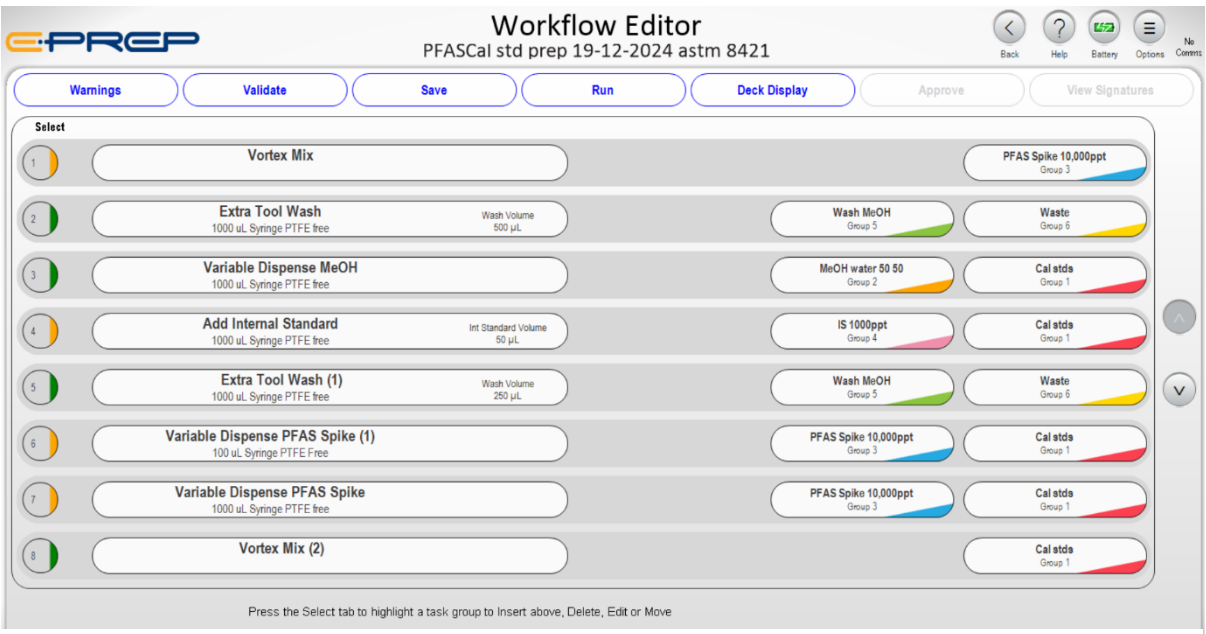


Figure 3 Workflow task list for creation of 6 x PFAS standards

METHODOLOGY

The process begins with thorough homogenization of calibration stock and internal standard solutions using a high precision vortex mixer operating at 1100 RPM for 30 seconds, guaranteeing uniform distribution throughout the sample matrix prior to aliquoting.

The system employs specialized PTFE-free syringes to eliminate potential contamination sources. The 1000 µL primary syringe undergoes a rigorous cleaning protocol with methanol, utilizing optimized aspiration (50 µL/s) and dispensing (400 µL/s) rates to ensure pristine condition between transfers. This syringe precisely delivers calibration matrix volumes from 450 µL to 945 µL, followed by accurate 50 µL internal standard additions across all calibration levels.

A dedicated 100 µL PTFE-free syringe is used for PFAS stock solution dispensing for calibration levels 1-3 (5-50 µL), while the 1000 µL syringe is used for higher-volume additions for levels 4-6. Between each transfer, tool wash protocol using 250 µL methanol maintains both internal and external syringe cleanliness, preventing carryover. The workflow culminates in a final homogenization step at 1100 RPM for 60 seconds, producing LC-MS/MS-ready calibration standards with reproducibility and accuracy. Table 3 below shows details of volumes and concentration of PFAS calibration standard preparation.

Table 3 Calibration details ranging from 50 to 5000 ng/L

	Spike Cal Std (ng/L)	IS Cal Std (ng/L)	Total Volume (µL)	Spike stock Conc (ng/L)	Spike Conc Volume (µL)	IS stock Conc (ng/L)	IS Conc Volume (µL)
Cal Standard 1	50	500	1000	10000	5	10000	50
Cal Standard 2	200	500	1000	10000	20	10000	50
Cal Standard 3	500	500	1000	10000	50	10000	50
Cal Standard 4	1250	500	1000	10000	125	10000	50
Cal Standard 5	2500	500	1000	10000	250	10000	50
Cal Standard 6	5000	500	1000	10000	500	10000	50

RESULTS AND DISCUSSION

The graphs in Figure 5 and Figure 6 illustrate the R^2 values for PFAS compounds, providing a detailed assessment of the linearity of the calibration curves. On the x-axis, the calibration levels range from 50 ng/L to 5000 ng/L, representing the concentration of each PFAS compound across a broad dynamic range. The y-axis displays the corresponding area under the curve (AUC), which is directly related to the concentration of the compounds and used to assess their quantification. Method validation results are based on isotope dilution quantification with surrogate recoveries consistently within acceptable range (70-130%).

The linearity of the calibration curves is determined by the correlation between the calibration levels (x-axis) and the AUC values (y-axis). A well-defined, linear relationship between the x- and y-intercepts indicates strong correlation. The R^2 value, which quantifies the goodness of fit, is represented on each graph. An R^2 value of 0.99 and above indicates that the calibration points align closely with the regression line, showing an excellent fit to the linear model. It shows that the calibration standards exhibit strong linearity, with minimal deviation, further confirming the reliability and accuracy of the method across the calibration range.

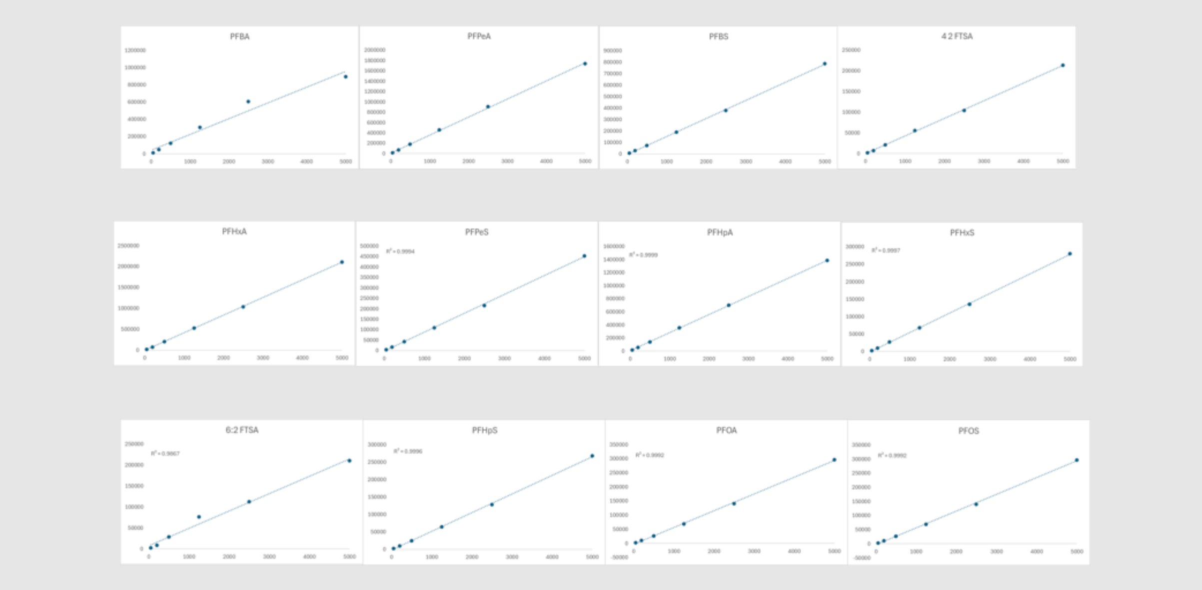


Figure 4 Calibration curve for PFAS compounds with R^2 values

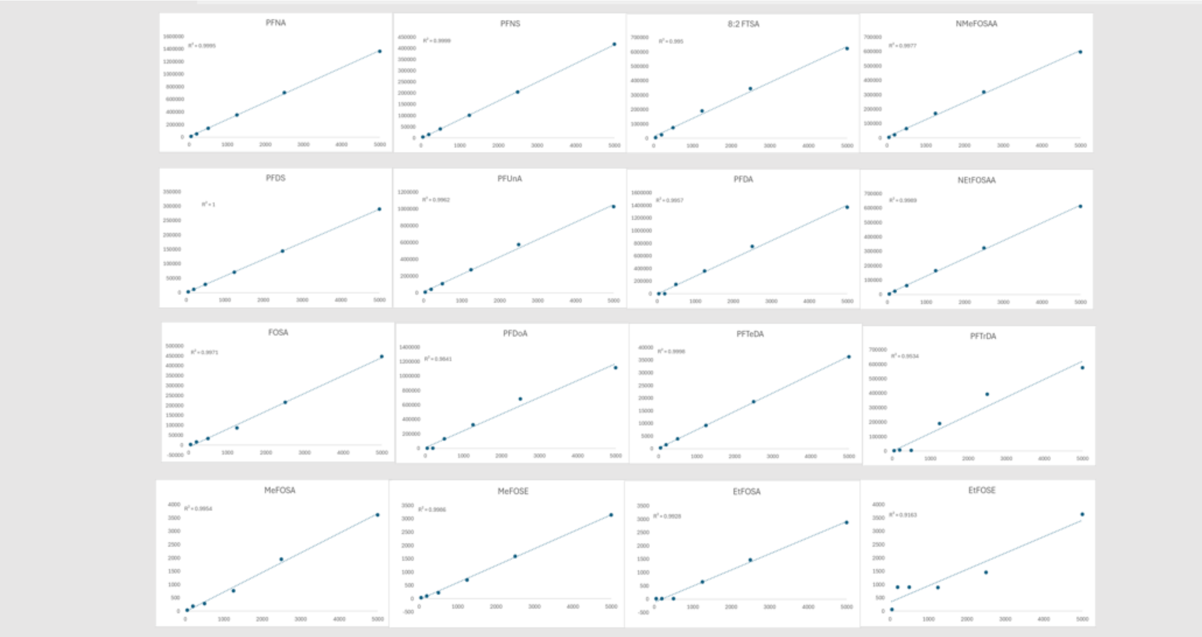


Figure 5 Calibration curve for PFAS compounds with R^2 values

Table 4 Data showing the R^2 value for 25 PFAS compounds for six calibration standards prepared using ePrep ranging from 50 ng/L to 5000 ng/L.

Number	PFAS Compound	R^2
1	PFBA	0.96
2	PFPeA	0.99
3	PFBS	0.99

4	4 2 FTSA	0.99
5	PFHxA	0.99
6	PFPeS	0.99
7	PFHpA	0.99
8	PFHxS	0.99
9	6 2 FTSA	0.98
10	PFDA	0.99
11	NEtFOSAA	0.99
12	FOSA	0.99
13	PFDoA	0.98
14	PFteDA	0.99
15	PFTrDA	0.95
16	MeFOSA	0.99
17	MeFOSE	0.99
18	EtFOSA	0.99
19	EtFOSE	0.91
20	PFUnA	0.99
21	PFDS	1
22	N-MeFOSAA	0.99
23	8:2 FTSA	0.99
24	PFNS	0.99
25	PFNA	0.99

CONCLUSION

The ePrep ONE Workstation delivers automated preparation of PFAS calibration standards for ASTM 8421 from 50 to 5000 ng/L into a 1.5mL autosampler vial with consistent R^2 values of 0.99 and above across compound classes. The system's contamination-free liquid handling achieves reliable calibration curves while eliminating manual intervention. The system demonstrates significant advantages including minimized reruns, reduced solvent consumption, and optimized staff utilization. These benefits directly support laboratory goals of increased throughput, cost optimization, and enhanced method sustainability. While this application note focuses on calibration standards, the ePrep ONE's versatility extends to sample preparation and integrated workflows combining both samples and standards. This validated performance confirms the system's effectiveness as a comprehensive solution for automated analytical sample preparation.

APPENDIX 1

Part numbers of the ePrep, accessories and spares used in this workflow

Description	Part Number
EPrep ONE Sample Preparation Workstation	01-01000
ePrep Probe Dispenser	01-04300
100 µL syringe (PTFE free)	01-09046
1000 µL syringe (PTFE free)	01-09047
2P vortex mixer	01-04100
Adapter plate	01-03006
Agilent Rack Capacity 54 vials Vial volume 2 ml	5022-6502
Reagent Jar Adapter Plate supplied with 5 X50 ml Reagent Jars	01-03085