# USE OF AUTOMATED SAMPLE PREPARATION IN ISO 17025 ACCREDITATION

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## Introduction

The process of obtaining accreditation under the ISO 17025 International Laboratory Standard involves validation of the methods used by the laboratory seeking accreditation. The current Standard was adopted in 2005. The goal is to put in place a Quality System for laboratories. The method validation aspect of this system requires writing of Standard Operating Procedures for method validation and for the various sample preparation and analysis techniques. Persistent Organic Pollutants (POPs) which have been regulated under the Stockholm Convention (in force since 2004) include polychlorinated dibenzo-p-dioxins and furans (PCDD/Fs) and polychlorinated biphenyls (PCBs). There is continued interest in these compounds because of their toxicity and in many industries strict environmental regulations apply. Sample cleanup and analysis when done manually can involve a number of days and is error-prone. Automating the sample preparation process can greatly help in getting the accreditation as fast as possible. Automation using our TotalPrep will, among others, result in faster turn around time of samples, lower and control the cost of analysis, and improve the quality of the data generated as part of method validation resulting in faster accreditation.[1] In this paper we will discuss results of method validation of serum during ISO 17025 accreditation using automated methods.

## Material and methods

### Pressurized Liquid Extraction (PLE), Figure 1.

10.7 g of NIST-1958 Fortified Human Serum was treated with 0.5 g of formic acid per mL. It was then mixed with 10 g of Hydromatrix (inert material) for drying. The sample mixture was placed in a stainless steel extraction cell (40 mLs volume), spiked with $^{13}C$ labeled isotope dilution standard, and capped at both ends with disposable Teflon end caps. The caps have metal frits to prevent possible blocking by particles. The cell was then placed in the extraction cell holder. The sample mixture was extracted with a 50%/50% mixture of dichloromethane and hexane at 120 $^{o}C$ and a pressure of 1500 psi for a total of 5 min of pre-heating, 20 min of heating and 20 min cool down. Cool down was achieved rapidly by 2 cooling fans. After cool down the cells were flushed with the 50%/50% extraction solvent mix and then with nitrogen to ensure complete capture of all pertinent analytes into collection tubes. Extraction data (pressure and temperature) were recorded by computer software and can be archived for retrieval purposes. Note that the use of Pressurized Liquid Extraction greatly reduced the time required for complete sample extraction to less than one hour.

### Solvent Evaporation/Volume Reduction, Figure 1.

Although with this serum sample only one extraction cycle was run, with larger volume extraction cells and multiple cycles a large volume of solvent containing the analytes can be collected. For subsequent multi-column automated sample clean up (below) a volume reduction to a few mLs is desirable. With our 6-position SuperVap Concentrator using a temperature controlled metal heating block samples can blown down in a dry, waterless fashion. The evaporation rate is increased by a gentle stream of nitrogen. The Concentrator automatically senses when the final sample volume has been reached and then shuts off the nitrogen stream. If this were the final step of the sample processing (in this case it was not), the concentrated samples could have been transferred manually into sample vials or automatically with direct-to-vial connections that can be attached to the collection tubes. In this study the sample was solvent-exchanged to hexane in the final stages of the blow down for the next clean up step.

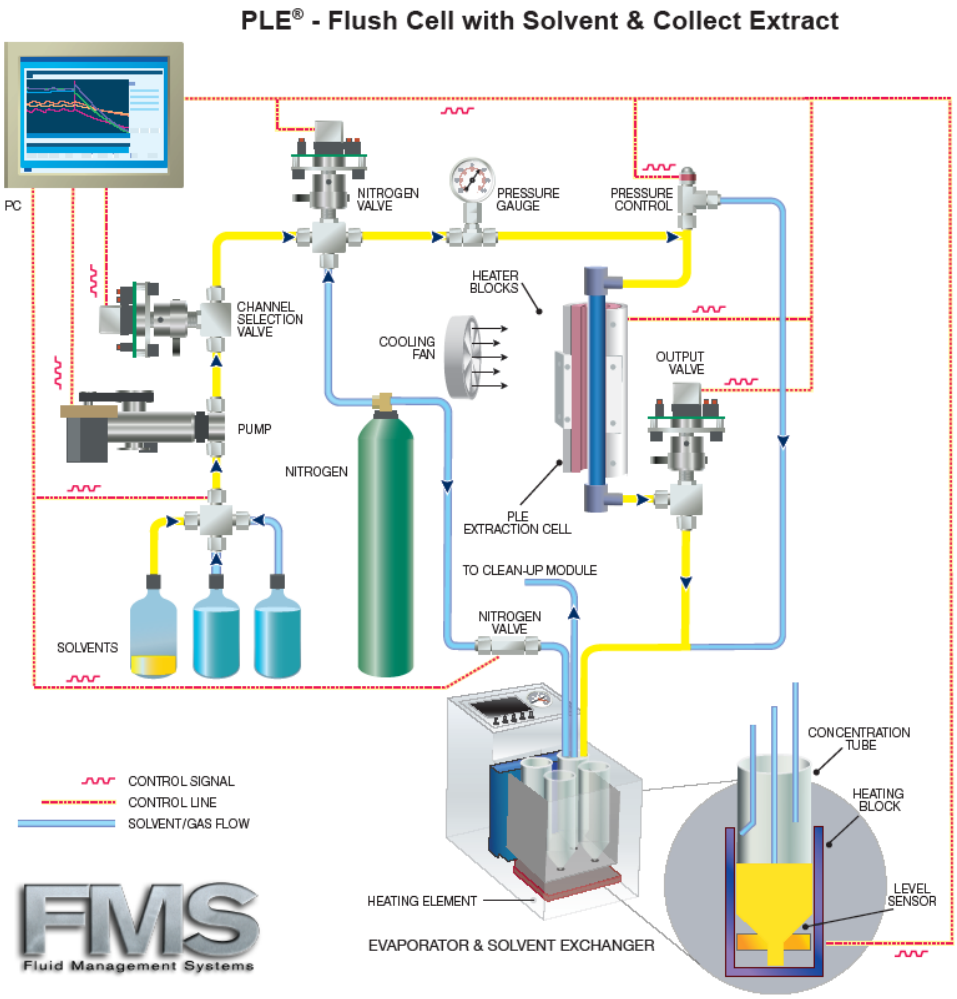
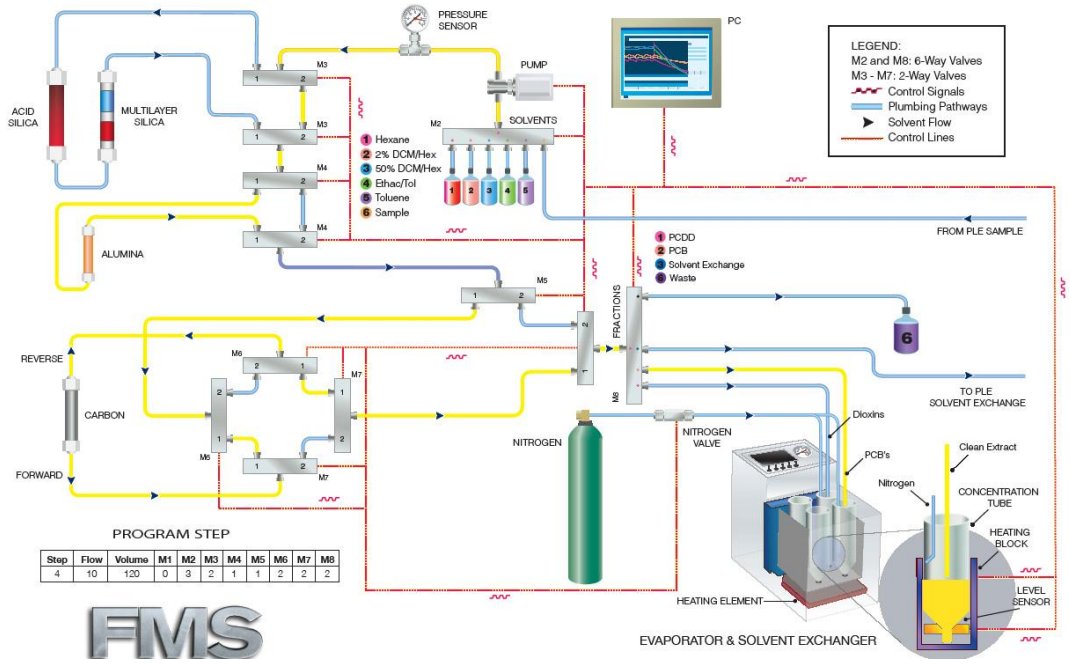



**Figure 1.** PLE schematic used in serum extraction.

**Clean up and Fractionation, Figure 2.**
The serum sample was cleaned up using our automated PowerPrep pre-packaged chromatographic column system. The system consists of a control module, valve modules, pump modules, and sample processing modules. The system used was operated via a PC; however, our new generation PowerPreps is stand alone with the computer control built into it with touch screen operation. A total of 4 columns were used: high capacity jumbo acid silica; classical acid-base silica; alumina; and carbon. The columns were pre-packaged by FMS. Solvents used for the sample clean up were hexane, 2% dichloromethane /98% hexane, 50%/50% dichloromethane: hexane, 50%/50% ethyl acetate/benzene, and toluene. The columns were pre-conditioned with the various solvents. Flow rates varied from 5-10 mLs/min. The number of steps involved varies with the analytes; in the case of collecting PCDD/Fs around 25 steps are typically programmed into the system. About half of these are prior to sample loading for purpose of conditioning the columns. The planar PCDD/Fs and co-planary PCBs (although not analyzed here) went through the entire process and were eluted in the last step off the carbon column with toluene. The entire clean up program ran for about 90 min.




| Step | Flow | Volume | M1 | M2 | M3 | M4 | M5 | M6 | M7 | M8 |
|---|---|---|---|---|---|---|---|---|---|---|
| 4 | 10 | 120 | 0 | 3 | 2 | 1 | 1 | 2 | 2 | 2 |

**Figure 2.** Schematic of PowerPrep automated multi-colum clean up system.

After the clean up step the sample was reduced to 0.5 mLs and then transferred to a sample vial for analysis. A Thermo Scientific DFS High Resolution GC/MS System at 10,000 resolution was used for reliable analysis, identification and quantification of the analytes. A DB-5 60 m x 0.25 mm column ID x 0.25 um phase thickness was used with a temperature program going from 130 $^{o}$C as initial temperature to a final temperature of 300 $^{o}$C in about 55 min. The analysis was carried out in accordance with the US EPA 1613 method.

**Results and discussion**

The data is shown in Table 1. As can be seen the serum analysis showed excellent agreement between the values found with our extraction and clean up method and the acceptable reference values provided for this fortified human serum. The concentrations found were in the low ppt range demonstrating the sensitivity of this method. After carrying out the analysis the method had been validated and was presented for accreditation. The whole process of extraction, clean up and analysis by properly trained personnel can be carried out in one day, resulting in same-day data collection from start to finish. If a larger number of samples is processed – up to six samples can be processed with a 6-position PLE and 6-positon PowerPrep - the entire procedure may take up to 24h including overnight analysis of the samples and quantification. This is a very good alternative to manual sample extraction using the Soxhlet-technique which typically takes up to 24h, followed by manual column chromatography clean up. The experimental (laboratory) part of obtaining ISO 17025 accreditation can greatly benefit from automated sample prep as illustrated by the method validation for human serum described here.

**Table 1 PCDD/F concentrations analyzed and reference values for NIST-1958 Fortified Human Serum Reference Material.**

| Analyte | Analyzed value in ppt | Acceptable (reference) value in ppt |
|---|---|---|
| **Analyte(s)** | | |
| **Dioxins** | | |
| 2,3,7,8-TCDD | 0.084 | 0.097±0.048 |
| 1,2,3,7,8-PeCDD | 0.091 | 0.11±0.055 |
| 1,2,3,4,7,8-HxCDD | 0.10 | 0.099±0.049 |
| 1,2,3,6,7,8-HxCDD | 0.27 | 0.36±0.18 |
| 1,2,3,7,8,9-HxCDD | 0.090 | 0.10±0.050 |
| 1,2,3,4,6,7,8-HpCDD | 0.41 | 0.59±0.29 |
| OCDD | 1.54 | 2.75±1.37 |
| **Difurans** | | |
| 2,3,7,8-TCDF | 0.097 | 0.11±0.055 |
| 1,2,3,7,8-PeCDF | 0.083 | 0.11±0.055 |
| 2,3,4,7,8-PeCDF | 0.18 | 0.22±0.11 |
| 1,2,3,4,7,8-HxCDF | 0.086 | 0.10±0.05 |
| 1,2,3,6,7,8-HxCDF | 0.089 | 0.11±0.055 |
| 1,2,3,7,8,9-HxCDF | 0.088 | 0.10±0.05 |
| 2,3,4,6,7,8-HxCDF | 0.67 | 0.96±0.48 |
| 1,2,3,4,6,7,8-HpCDF | 0.20 | 0.31±0.15 |
| 1,2,3,4,7,8,9-HpCDF | 0.12 | 0.086±0.043 |
| OCDF | 0.13 | 0.089±0.044 |

**References**:

1. Focant JF, Shirkhan H, Patterson Jr DG. (2009) *Organohalogen Cmpds* 71: 2438-2443.